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Office of Water
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Methods for Evaluating Wetland Condition

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#18 Biogeochemical Indicators

Major Contributors

University of Florida, Institute of Food and Agriculture,
Soil and Water Science Department

K. Ramesh Reddy and Mark W. Clark

Prepared jointly by:

The U.S. Environmental Protection Agency
Health and Ecological Criteria Division (Office of Science and Technology)

and

Wetlands Division (Office of Wetlands, Oceans, and Watersheds)

Notice

The material in this document has been subjected to U.S. Environmental Protection Agency (EPA) technical review and has been approved for publication as an EPA document. The information contained herein is offered to the reader as a review of the "state of the science" concerning wetland bioassessment and nutrient enrichment and is not intended to be prescriptive guidance or firm advice. Mention of trade names, products or services does not convey, and should not be interpreted as conveying official EPA approval, endorsement, or recommendation.

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This entire document can be downloaded from the following U.S. EPA websites:

http://www.epa.gov/waterscience/criteria/wetlands/

http://www.epa.gov/owow/wetlands/bawwg/publicat.html

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Foreword

In 1999, the U.S. Environmental Protection Agency (EPA) began work on this series of reports entitled *Methods for Evaluating Wetland Condition.* The purpose of these reports is to help States and Tribes develop methods to evaluate (1) the overall ecological condition of wetlands using biological assessments and (2) nutrient enrichment of wetlands, which is one of the primary stressors damaging wetlands in many parts of the country. This information is intended to serve as a starting point for States and Tribes to eventually establish biological and nutrient water quality criteria specifically refined for wetland waterbodies.

This purpose was to be accomplished by providing a series of "state of the science" modules concerning wetland bioassessment as well as the nutrient enrichment of wetlands. The individual module format was used instead of one large publication to facilitate the addition of other reports as wetland science progresses and wetlands are further incorporated into water quality programs. Also, this modular approach allows EPA to revise reports without having to reprint them all. A list of the inaugural set of 20 modules can be found at the end of this section.

This last set of reports is the product of a collaborative effort between EPA's Health and Ecological Criteria Division of the Office of Science and Technology (OST) and the Wetlands Division of the Office of Wetlands, Oceans and Watersheds (OWOW). The reports were initiated with the support and oversight of Thomas J. Danielson then of OWOW, Amanda K. Parker and Susan K. Jackson (OST), and seen to completion by Ifeyinwa F. Davis (OST). EPA relied on the input and expertise of the contributing authors to publish the remaining modules.

More information about biological and nutrient criteria is available at the following EPA website:

http://www.epa.gov/ost/standards

More information about wetland biological assessments is available at the following EPA website:

http://www.epa.gov/owow/wetlands/bawwg

LIST OF "METHODS FOR EVALUATING WETLAND CONDITION" MODULES

Summary

This module discusses biogeochemical parameters and their use as indices to characterize the nutrient status of wetlands. Biogeochemical processes in the soil and water column drive key ecosystem functions associated with wetland values (e.g. water quality improvement through denitrification, long-term nutrient storage in the organic matter, etc.). Process level measurements reflect the functionality of a wetland and potential impairment due to impacts; however, these measurements are often tedious and costly. Instead, it is possible to develop a relative measure of process rates and potential by evaluating components of biogeochemical cycles that are either end products or sources of material for a given process. In the case of many processes within the carbon (C), nitrogen (N), phosphorus (P) or sulfur (S) cycles, microbial communities mediate the rate and extent of these reactions in soil and the water column. As a result, biogeochemical indicators associated with these processes often respond rapidly to perturbations and are spatially restricted to the impact zone. These indicators will persist over moderate time scales and in the absence of standing water. In the discussion that follows, we will introduce a suite of basic and more advanced biogeochemical indicators that can be used to characterize wetland condition and evaluate those indices. We will also examine indices that have been most sensitive to nutrient impact in the Florida Everglades.

Purpose

The purpose of this module is to describe biogeochemical indicators that can be used to monitor nutrients in wetland systems in an ecologically meaningful and scientifically rigorous manner.

Introduction

Many species depend upon wetlands for successful completion of their life cycle and most require, or benefit from, nearby aquatic habitat. Changes in the structure and function of a wetland will eventually have far-reaching effects on the biota of the wetland and the surrounding uplands. Monitoring wetland systems can provide information on environmental change, including changes in community structure and function, in both the wetland and adjacent upland watershed.

Development of sound concepts and methodologies for ecosystem monitoring require an understanding of ecosystem structure and function. Given the high cost of environmental monitoring in terms of time, human resources and funding, methods need to be developed that are simple, efficient, scientifically rigorous and ecologically meaningful. One of the most attractive approaches to developing scientifically rigorous methods is based on the concept of using physical, chemical or biological properties or processes as indicators of wetland condition, change or response to anthropogenic impacts.

Wetlands host complex microbial communities, including bacteria, fungi, protozoa, and viruses. The size and diversity of microbial communities are directly related to the quality and the quantity of resources available in the system. Many of the water and soil parameters that influence the ecosystem are the end products of biogeochemical processes that are microbiologically mediated. Microbial processes and populations often have more rapid turnover times than higher trophic stages. Due to their large size, they are often more responsive to environmental changes at lower thresholds. Microbial processes are potentially very sensitive to perturbations such as external nutrient loading, hydrologic alterations, and fire. These characteristics make them efficient indicators of wetland conditions.

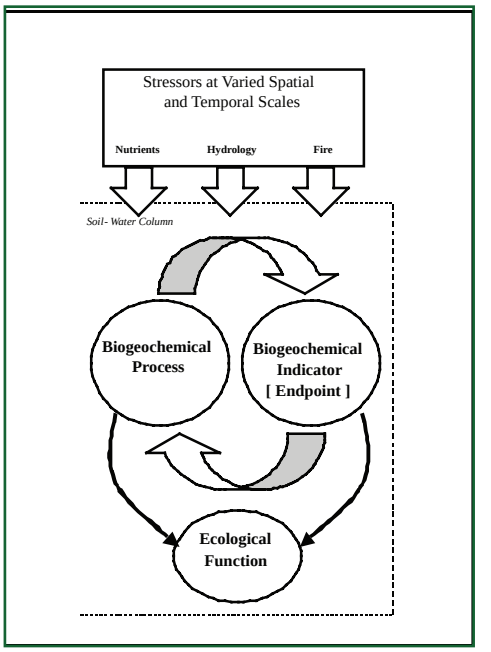

FIGURE 1: SCHEMATIC SHOWING THE LINKAGE BETWEEN BIOGEOCHEMICAL PROCESSES AND INDICATORS.

Biogeochemical processes are also likely to be highly reliable indices in the sense that ecological changes at such a fundamental level will affect all species utilizing the ecosystem. Changes at higher levels, such as a decline in populations of a suite of higher organisms may be due to factors that affect only a small portion of the biota, whereas changes in biogeochemical processes signify comprehensive alteration of the biota. Thus, when describing the structure and function of an ecosystem, it is critical to evaluate the water and soil quality using an integrated framework that links processes and associated indicators. Biogeochemical processes may be sensitive and reliable indicators of wetland condition, but their measurement can be time-consuming and expensive. However, concentrations of certain chemical substrates, intermediates, and end products of ecologically potent, biogeochemical processes may provide rapid and inexpensive indicators of the rates of those processes. Hence, simple measurements of biogeochemical processes in wetlands could be extremely efficient indicators of the wetland and the entire watershed. Furthermore, relationships between indicators and processes may provide a more reliable estimate of ecosystem health for assessment at a landscape level.

A fundamental understanding of the biogeochemical processes regulating the functions of the ecosystem is critical to evaluating nutrient impacts and successes of restoration efforts. The certainty associated with an assessment decreases if the factors that affect biogeochemical processes regulating the fate and transport of nutrients in wetlands are not well understood; i.e., the risk assessment is only as good as the information/knowledge available at the time. Therefore, it is

imperative that sound linkages are developed between nutrient indicator parameters and assessment of structural and functional impacts.

Eutrophication of wetlands can be attributed to: (i) increased external inputs of nutrients from point and non-point sources; and/or, (ii) accelerated nutrient cycling within the soil associated with change in environmental conditions of the soil and water column. Eutrophication is often linked to only external sources of nutrients in many wetlands. However, internal nutrient sources can be equally important, especially in highly impacted wetlands or older wetlands with large reserves of organic and inorganic bound nutrients (Fisher and Reddy 2001).

Anthropogenic nutrient loading from point or non-point sources to a nutrient-limited wetland system can alter physical, chemical and biological properties and processes in the soil and water that in turn can influence ecosystem function and productivity (Figure 1). Wetlands, as low-lying areas in the landscape, receive inputs from all hydrologically connected uplands. Many wetlands are open systems receiving inputs of carbon (C) and nutrients from upstream portions of the watershed including agricultural and urban areas. Prolonged nutrient loading to wetlands can result in distinct gradients in floodwater and soil. Mass loading and hydraulic retention time determine the degree and extent of nutrient enrichment. The continual nutrient loading in an oligotrophic wetland results in two distinct zones: (i) a zone of high nutrient availability or non-limiting nutrient conditions near the input; and, a (ii) a zone of low nutrient availability or nutrient limiting conditions furthest from the input point. Between these two extremes, there exists a gradient in quality and quantity of organic matter, nutrient accumulation, microbial and macrobiotic communities, composition, and biogeochemical cycles. This enrichment effect can be seen in many freshwater wetlands, most notably in the subtropical Everglades (Davis 1991, Reddy, et al.1993; Craft and Richardson 1993 a, b; DeBusk, et al.1994).

Low-nutrient systems are characterized by low external loading of nutrients and relatively closed, efficient elemental cycling (Odum 1969, 1985). Nutrients are held in tight, closed cycles, whose efficiency enables maintenance of energy flow. Hence, microbial activity and plant productivity are nutrient limited. In response, wetland vascular plants, periphyton, and microbial communities are extremely efficient in utilizing and conserving nutrients. Plant detritus in low-nutrient wetlands generally has high C:N:P mass ratios. The overall turnover rate of high C:N:P ratio organic matter is usually slow, and long-term decomposition may be both carbon and nutrient limited (Davis 1991; DeBusk and Reddy 1998). Decomposition of high C:N:P plant detrital material results in conditions where microbial and periphytic communities out-compete vascular plants for nutrients. However, environmental factors such as water-table fluctuations and fire can result in pulsed release of nutrients, which may provide a significant, although temporally infrequent, source of plant- available nutrients in low- nutrient systems (Lodge, et al.1994).

High-nutrient wetland systems are characterized by rapid turnover of C and nutrients, and by open elemental cycling, where nutrient inputs often exceed demand. These systems are low nutrient stressed and

contain varying degrees of internal cycling. In response, vascular plants and microbial/ periphyton communities are less efficient in nutrient utilization. Plant detritus in impacted areas generally has low C:N:P mass ratios, and high net mineralization. The release of nutrients during decomposition results in decreased importance of internal cycling by microbes and plants as compared with nutrient loading from external sources.

One of the most attractive approaches to measure and quantify nutrient availability is based on the concept of using physical, chemical or biological properties or processes in the soil and water column as indicators of change or response to anthropogenic impacts. In this report, we describe easily measurable indicators with reasonable scientific rigor and reliability that will assess nutrient conditions in wetlands, then suggest a subset of indicators that are most responsive and sensitive to be used as endpoints for assessment of impacts. As defined, assessment endpoints are explicit expressions of an environmental value to be protected, while measurement endpoints are measurable responses of an assessment endpoint to a stressor (USEPA 1992; Suter, 1990).

In this module, we describe two levels of indicators;(i) Level I indicators which are easily measurable; and (ii) Level II indicators which provide more scientific rigor and are used to support easily measurable indicators. Only select Level I indicators will be promoted as indicator endpoints for assessment of impacts.

NUTRIENT CYCLE IN WETLANDS

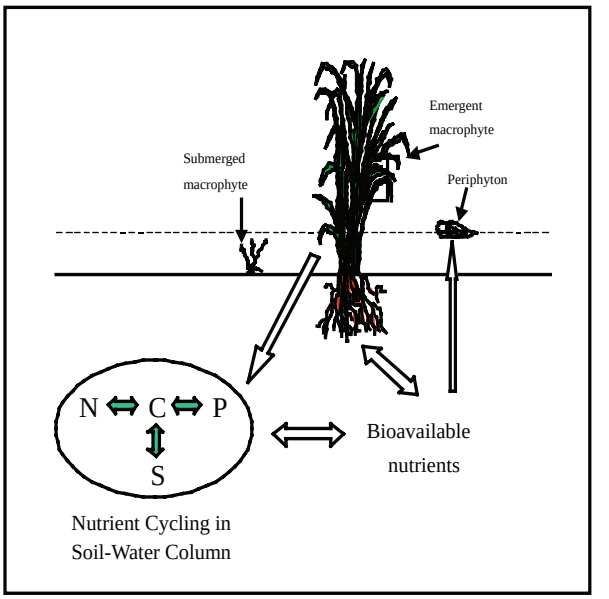

FIGURE 2: SCHEMATIC SHOWING BASIC NUTRIENT CYCLES IN SOIL-WATER COLUMN OF A WETLAND.

Characteristics of the Soil and Water Column

Aerobic-anaerobic interfaces in the water column (e.g. surfaces of detrital plant tissue and benthic periphyton mats), at the soil-water interface, and in the root zone of aquatic macrophytes are unique features of wetlands, as compared to upland landscapes. We define the soil-water column, as soil with overlying floodwater. The juxtaposition of aerobic and anaerobic zones in wetlands supports a wide range of microbial populations and associated metabolic activities, with oxygen-reduction occurring in the aerobic interface of the

substrate, and reduction of alternate electron acceptors occurring in the anaerobic zone (Reddy and D'Angelo 1994, 1996). Under continuously saturated soil conditions, vertical layering of different metabolic activities can be present, with oxygen-reduction occurring at and just below the soil–floodwater interface. Much of the aerobic decomposition of plant detritus occurs in the water column; however, the supply of oxygen may be insufficient to meet demands and drive certain microbial groups to utilize alternate electron acceptors, e.g., nitrate, oxidized forms of Fe and Mn, sulfate and HCO_3.

Soil drainage adds oxygen to the soil, while other inorganic electron acceptors may be added through hydraulic loading to the system. Draining wetland soil accelerates organic matter decomposition due to the introduction of oxygen deeper into the profile. In many wetlands, the influence of NO_3^-, and oxidized forms of Mn and Fe on organic matter decomposition is minimal, as the concentration of these electron acceptors is usually low. The demand for electron acceptors of greater reduction potentials (NO_3^-, Fe and Mn) is high and they are depleted rapidly from the system. Long-term sustainable microbial activity is then supported by electron acceptors of lower reduction potentials (sulfate and HCO_3). Methanogenesis is often viewed as the terminal step in anaerobic decomposition in freshwater wetlands, whereas sulfate reduction is viewed as the dominant process in coastal wetlands. However, both processes can function simultaneously in the same ecosystem and compete for available substrates.

A simple way to characterize wetlands for aerobic and anaerobic zones is to determine the oxidation-reduction or redox potential (Eh) of the soil-water column. The redox potential is expressed in units of millivolts (mV) and is measured using a voltmeter. Typically, wetlands with Eh values >300 mV are considered aerobic and exhibit drained soil conditions, while soils with Eh values <300 mV are considered as anaerobic and are devoid of molecular oxygen.

Basic Elemental Cycles

CARBON

Compared to upland systems, most wetland ecosystems show an accumulation of organic matter, and therefore wetlands function as global sinks for carbon. Accumulation of organic (C) in wetlands is primarily a result of the balance of two processes—C fixation through photosynthesis and C losses through decomposition. Rates of photosynthesis in wetlands are typically higher than other ecosystems, and rates of decomposition are typically lower due to anaerobic conditions, hence organic matter tends to accumulate. In addition to maintaining proper functioning of wetlands, organic matter storage also plays an important role in regulating other ecosystems and the biosphere. For example, up to 55% of organic matter contains variable amounts of nutrients N, P, S, O, and H. Therefore, the accumulation of organic matter in wetlands prevents transport of these nutrients to downstream aquatic systems.

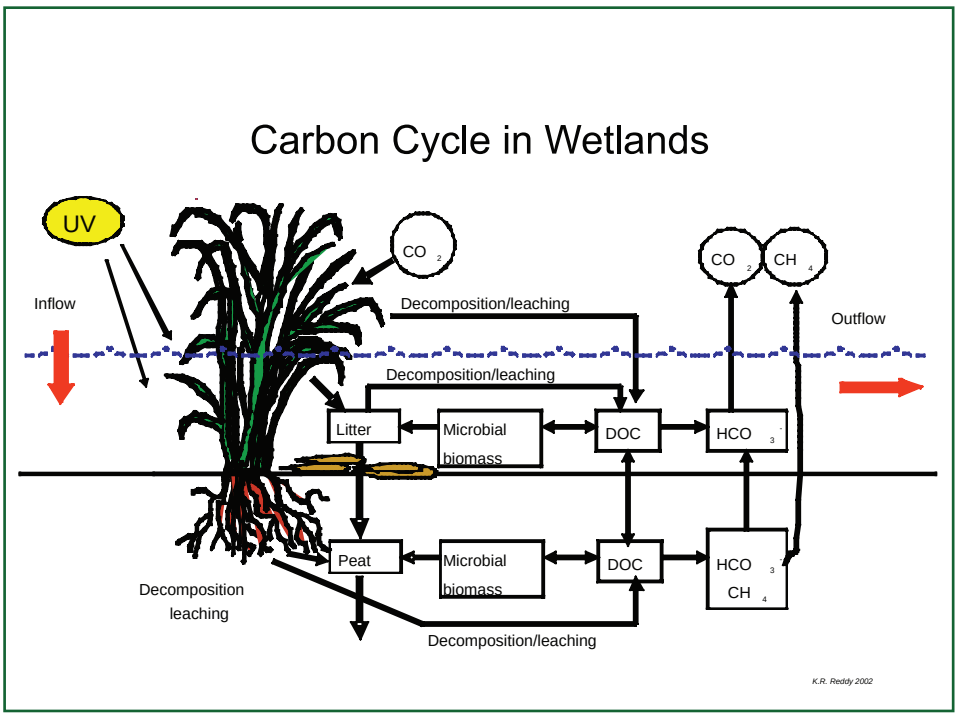

FIGURE 3: SCHEMATIC SHOWING BASIC CARBON CYCLES IN THE SOIL-WATER COLUMN OF A WETLAND.

Major pools of C storage include soil organic matter, detrital organic matter, microbial biomass, plant biomass, and dissolved organic carbon. Microbial biomass accounts for <5% of the total C in the soil and water column. The dominant constituents of soil organic matter include cellulose, hemicellulose, and lignin. The cellulose and hemicellulose are readily biodegradable, while lignin is highly stable under anaerobic conditions. Carbon compounds recalcitrant to aerobic and anaerobic decomposition tend to accumulate in wetlands as either undecomposed plant tissues (peat) or humic substances. Formation of humic substances probably involve condensation reactions between reactive phenolic groups of tannins and lignins with water soluble nonhumic substances, which is catalyzed by phenoloxidase enzymes in soils (Francois 1990). This mechanism accounts for the large molecular weight and heterogeneous humic substances that contain significant amounts of N, P, S in their structures. In the absence of oxygen, humic substances are resistant to decomposition, and represent a significant carbon and nutrient storage in wetlands. Under drained conditions, humic substances are more readily degraded, which releases nutrients to the bioavailable pool, thereby affecting downstream water quality.

Although the loading of anthropogenic nutrients stimulates the growth of aquatic vegetation in wetlands, a significant por-

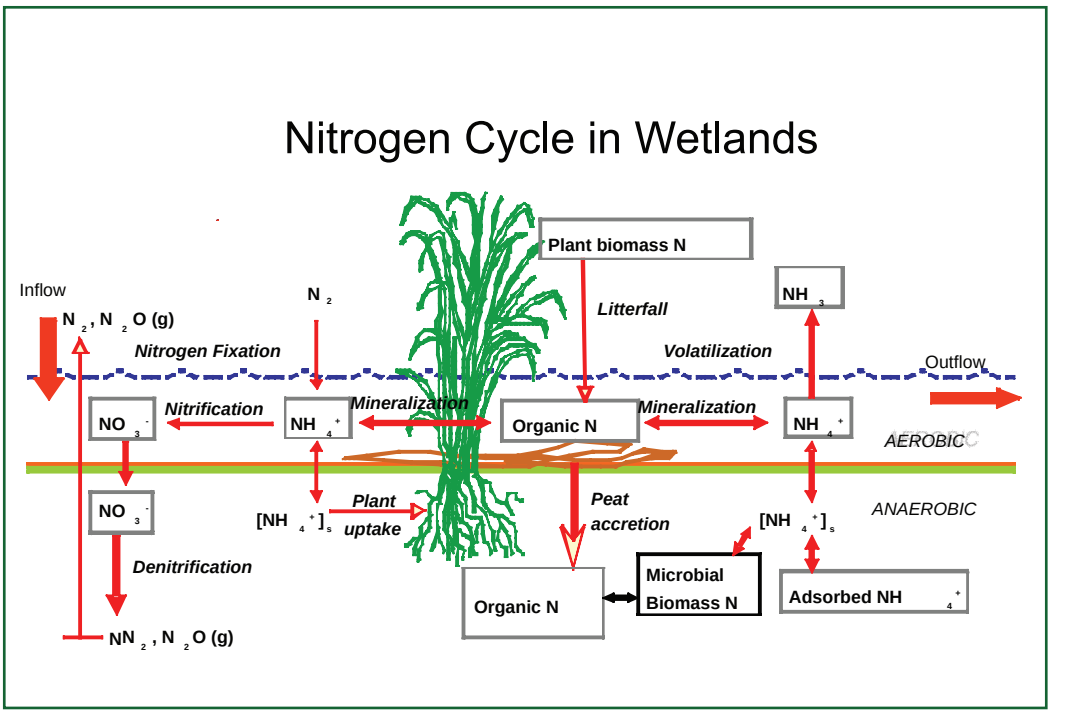

FIGURE 4: SCHEMATIC SHOWING BASIC NITROGEN CYCLES IN THE SOIL-WATER COLUMN OF A WETLAND.

tion of the nutrient requirements may be met through remineralization during decomposition of organic matter. The rate of organic matter turnover and nutrient regeneration is influenced by: (i) hydroperiod (Happell and Chanton 1993); (ii) characteristics of organic substrates, (Webster and Benfield 1986; Enriquez, et al.1993; DeBusk and Reddy 1998); (iii) supply of electron acceptors (D 'Angelo and Reddy 1994a, b); and, (iv) addition of growth limiting nutrients (Button 1985; McKinley and Vestal 1992; Amador and Jones 1995). For this reason both internal and external factors can affect carbon cycling and nutrient release from organic matter in wetlands.

NITROGEN

Nitrogen (N) enters wetlands in organic and inorganic forms. The relative proportion of each depends on source and type of water entering these systems. Organic forms are present in dissolved and particulate fractions, while inorganic N (NH_4-N, NO_3-N and NO_2-N) is present in dissolved fractions. Particulate fractions are removed through settling and burial, while the removal of dissolved forms is regulated by various biogeochemical reactions functioning in the soil and water column. Relative rates of these processes are affected by physico-chemical and biological characteristics of soils, organic substrates, and water column.

Nitrogen reactions in wetlands effectively process inorganic N through nitrification and denitrification, ammonia volatilization and plant uptake. These processes aid in lowering levels of inorganic N in the water column. A significant portion of dissolved organic N assimilated by plants returns to the water column during breakdown of detrital tissue or soil organic matter, and the majority of this dissolved organic N is resistant to decomposition. Under these conditions, water leaving the wetlands may contain elevated levels of N in organic form. However, relative rates of these reactions will depend on the optimal environmental conditions present in soil and water column.

PHOSPHORUS

Wetlands regulate phosphorus (P) retention by physical mechanisms (sedimentation and entrainment) and biological mechanisms (uptake and release by vegetation, periphyton and microorganisms). Phosphorus in the influent water is typically in the form of soluble and particulate fractions, with both forms containing a certain proportion of inorganic and organic pools. The relative proportions of these pools depend on the source and the type of water that enters the system. For example, municipal wastewater may contain a large proportion (>75%) as inorganic P in soluble forms, as compared to effluents from agricultural watersheds where percentage of P loading is predominantly in the particulate fraction. Constructed and riparian (buffer) wetlands can function as effective sediment traps, as such, P associated with suspended sediments can be effectively removed by wetlands.

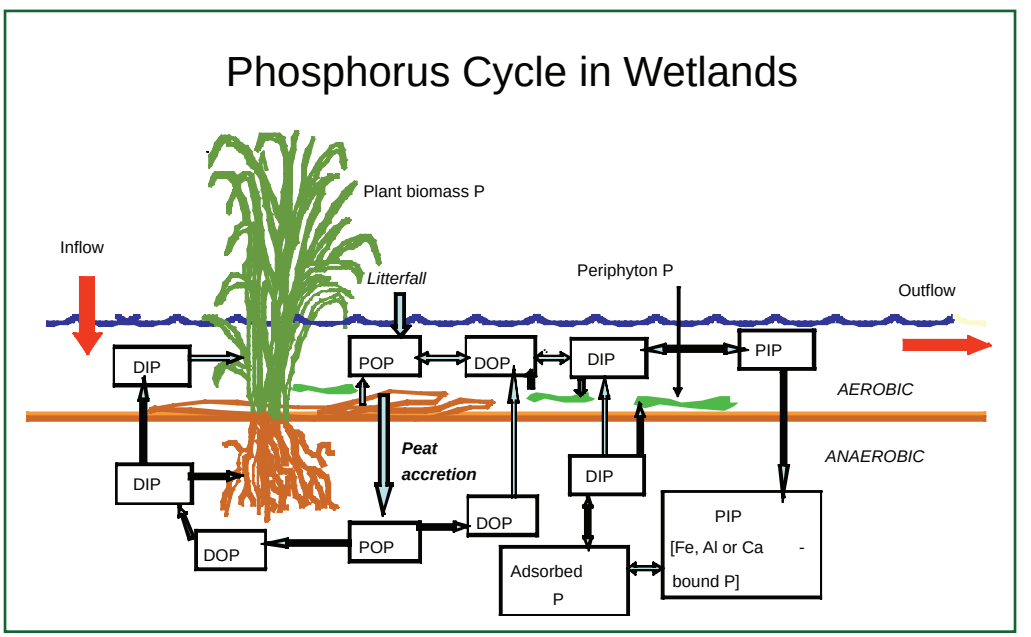

FIGURE 5: SCHEMATIC SHOWING BASIC PHOSPHOROUS CYCLES IN THE SOIL-WATER COLUMN OF A WETLAND.

Inorganic P — content of wetland soils vary with soil type. Typically, mineral wetland soils have a higher proportion of total soil P in inorganic fractions. Discrete fractions of inorganic P are determined using simple chemical fractionation schemes (Reddy, et al.1998a).

Porewater P — Phosphorus present in dissolved form in soil interstitial water. Phosphorus present in this pool is highly mobile and readily available for plant uptake or flux into the overlying water column. The concentration of P in soil porewater is regulated by soil physicochemical properties and the soil's capacity to adsorb or desorb P.

Exchangeable P — Inorganic P ions adsorbed on positively charged soil or organic matter surfaces. Under acid conditions, excessive protons may result in positive charges on soil particle surfaces. These positively charged surfaces can adsorb negatively charged phosphate ions. Phosphate sorbed on surfaces maintains equilibrium with phosphate ions in soil porewater.

Iron and Aluminum bound P — Iron and aluminum minerals can retain P by adsorbing on their surfaces or forming discrete minerals, such as ferric phosphate or aluminum phosphate. These minerals are stable under acid soil conditions.

Calcium and Magnesium bound P — In alkaline soils, inorganic P is typically bound to calcium or magnesium based minerals.

Residue P — This form of P is insoluble in either alkali or in acid. Phosphorus present in this pool is typically not bioavailable.

Organic P fraction in soils and sediments accounts for a high proportion of the total P, with this factor accounting for 20-60% of total P in mineral soils (Tiessen et al. 1994), 10-70% in lake sediments, and 40-90% in Histosols (Reddy, et al.1998a). Most of the organic P in soils is derived from plant detritus and synthesized in part by soil organisms from inorganic sources (Sanyal and DeDatta 1991). Organic P can be classified into three groups: (i) inositol phosphates, (ii) nucleic acids, and (iii) phospholipids (Anderson 1980), with inositol phosphates comprising up to 60% of the total soil organic P (Tate 1984; Turner 2002).

Water column P can be readily removed by periphyton and algal uptake, followed by deposition of dead biomass on the soil surface. Periphyton communities are effective sinks in low P systems. In treatment wetlands where P loading is usually high, the periphyton effect on P removal through direct assimilation may be minimal. Similarly, vegetative uptake can provide either short-term or long-term sink for P depending on type of vegetation present in the wetland. Phosphorus tied-up in detrital plant/algal tissue is rapidly released into the water column during decomposition. However, over long-term periods, significant portions of organic P may remain in the soil as a part of peat accumulation in wetlands. Under anaerobic conditions, these forms of P are relatively resistant to microbial breakdown, and can be considered an important P sink.

Inorganic P added to wetlands at concentrations considerably greater than those present in the soil porewater diffuses into the soil and is retained by oxides and hydroxides of iron and aluminum in acid soils, and by calcium carbonate in alkaline soils. In soils dominated by Fe oxides, P can be readily immobilized through sorption and precipitation by ferric oxyhydroxide, and formation of ferric phosphate in the oxidized zones at the soil-water interface. In calcareous systems, P can be precipitated as Ca mineral bound-P, especially when pH of the floodwater is altered diurnally by photosynthetic activities of algae. On the other hand, when the amount of soluble P in the soil porewater is higher than in the floodwater, the steep gradients produced result. in the diffusive flux of P from the soil into the overlying water column. The P released into water column is then either re-assimilated by planktonic organisms and precipitated as Ca-phosphate, or released downstream. In calcareous systems, co-precipitation of P with $CaCO_3$ is a dominant mechanism in immobilization of soluble P. In the water column where pH fluctuates diurnally, with high values during daytime (photosynthetic activity of algae) and low values during night (respiration by algae and bacteria), P can be precipitated and resolubilized (Diaz, et al.1994). The rate of adsorption is controlled by soil pH and Eh, adsorptive surface area (active iron and aluminum or calcium carbonate), and temperature. These regulators can be used as indicators to evaluate P retention capacity of wetland soils.

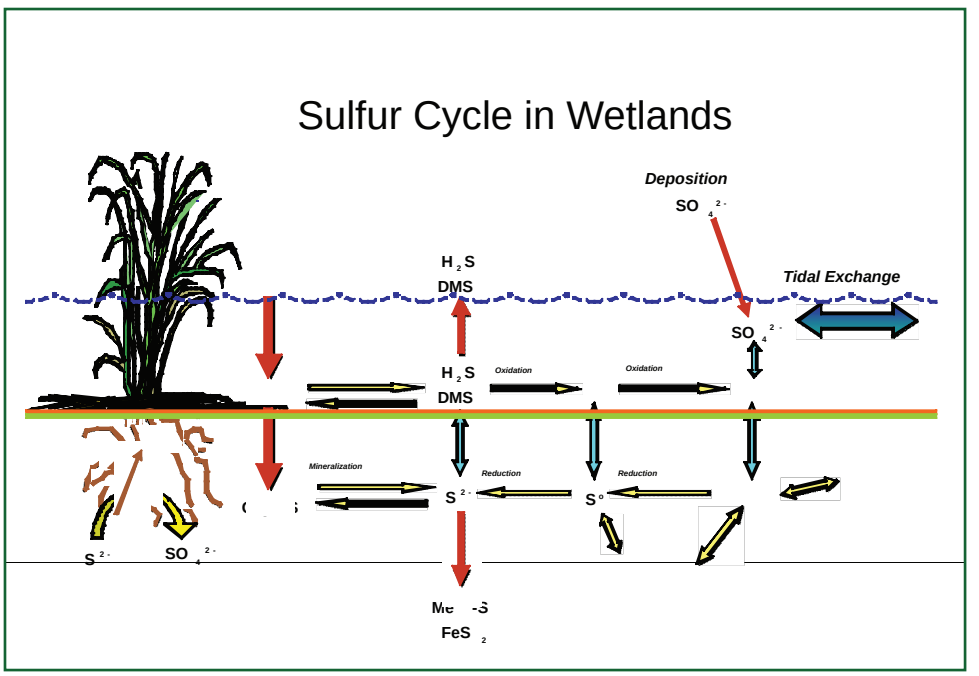

FIGURE 6: SCHEMATIC SHOWING BASIC SULFUR CYCLES IN THE SOIL-WATER COLUMN OF A WETLAND.

SULFUR

In freshwater wetlands, sulfur (S) cycling was assumed to play a minimal role in regulating decomposition and nutrient release. The role of S in coastal wetlands is well established. However, SO_4^{2-} inputs from adjacent watersheds to freshwater wetlands can increase the role of the S cycle; increased inputs into saltwater wetlands can accelerate the S cycle, especially sulfate reduction and associated microbial processes. Similar to C and N, organic S mineralization can be related to substrate quality, microbial biomass and extracellular enzyme activity. Likewise, SO_4^{2-} reduction can be directly related to dissolved organic C and fatty acids.

In estuarine wetland soils and in freshwater environments with appreciable SO_4^{2-} , microbial reduction of sulfate to S^- or H_2S occurs. The characteristic "rotten egg" odor of tidal and estuarine wetland soils is the result of H2S production by sulfate reducers. Numerous species of bacteria, including Desulfovibrio, Desulfobacter, Desulfococcus and others, can use SO_4^{2-} as a terminal electron acceptor (Ponnamperuma 1972; Widdel and Hansen 1992). In wetland soils and sediments, most SO_4^{2-} reducing bacteria are mesophiles, existing at optimum temperatures of 20 to 40°C (Wieder and Lang 1988; Widdel and Hansen 1992). Like iron (Fe) and manganese (Mn) reduction, SO_4^{2-} reduction may be inhibited by the presence of other terminal electron acceptors. Appreciable SO_4^{2-} reduction does not occur in the presence of oxidized Fe and Mn or NO_3 (Ponnamperuma 1972; Yoshida 1975) because Fe/Mn reducers and denitrifiers maintain the concentration of electron donors at concentrations that are too low to support populations of SO_4^{2-} reducers (Lovley 1991).

A major difference between freshwater and estuarine wetlands is the predominance of sulfate reduction over methanogenesis in estuarine wetlands. The concentration of SO_4^{2-} in most freshwater ecosystems is only 0.1-0.2 mM as compared to 20-30 mM in seawater (Capone and Kiene 1988). As such, increased SO_4^{2-} availability in estuarine wetlands leads to higher rates of sulfate reduction and additions of SO_4^{2-} to freshwater systems can change the dominant organic matter decomposition pathway from methanogenesis to sulfate reduction. (Castro and Dierberg 1987; Capone and Kiene 1988). Estimates of sulfate reduction in salt marsh sediments are on the order of 1000-2000 g S/m^2/yr with approximately 50-90% of the total organic matter decomposition occurring via this pathway (Howarth 1984; Howes, et al.1984; Howarth and Giblin 1983). In freshwater wetlands, sulfate reduction is generally limited by the availability of SO_4^{2-} (Nedwell 1984). Although sulfate reduction generally is lower in freshwater wetlands than in estuarine wetlands, significant sulfur inputs from acid deposition may lead to rates of sulfate reduction that are comparable to those measured in estuarine soils and sediments.

Reference Wetlands

To determine the nutrient impacts on wetlands, one must establish the background conditions in a wetland that is not impacted by nutrients. In addition to nutrients, impacts on wetlands occur from hydrologic fluctuations, fire, and management practices im-

posed on these systems. All these impacts can also influence the nutrient profiles in wetlands. Thus, it is critical to determine the background levels of biogeochemical indicators and processes that can be used to determine the change in wetland conditions. Wetland sites monitored for background levels will function as "reference" sites, which can be used to establish wetland condition. Reference sites for sampling and monitoring can be identified based on the historical information available for the site. One reference site for a given wetland type within the same watershed may be adequate. In many wetlands, it may be impossible to find sites that are not affected by anthropogenic impacts. Under these conditions, minimally impacted sites can be used as "reference" sites. Once the reference sites are established, spatial and temporal variability in selected indicators should be monitored to determine the ranges in values. This initial database is essential to establish nutrient criteria.

In certain watersheds, the whole wetland may be impacted and reference sites may not be available. These wetlands typically have high accumulations of organic matter. For these sites, native soil nutrient content can be used as background condition. This can be accomplished by taking intact soil cores and determining the nutrient profiles. Typically, impacted wetlands will have high nutrient levels in surface layers and decrease with depth, and reach steady levels at lower soil depths. Nutrient levels in lower soil depths can be used as an indication of background levels for that site. Depth of nutrient impact can also be estimated by determining the age of the material using Cs-137 dating techniques (Reddy, et al.1993; Ritchie and McHenry 1990).

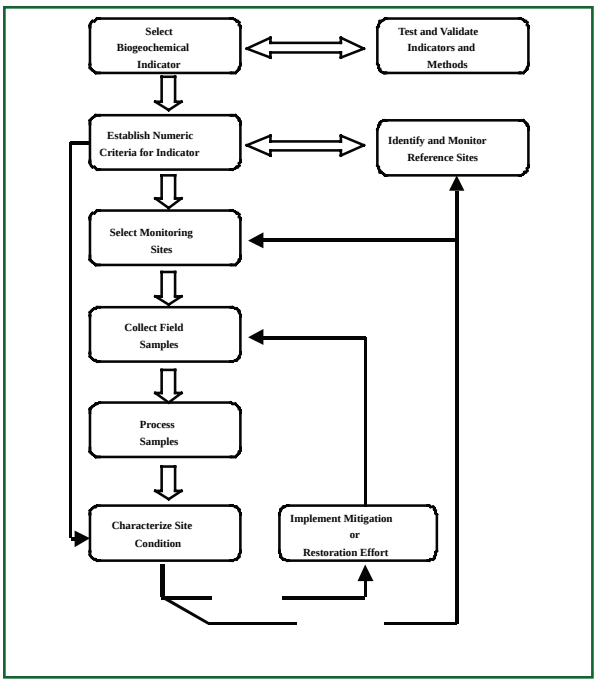

FIGURE 7: SCHEMATIC SHOWING THE PROPOSED STEPS TO DEVELOP AND EVALUATE BIOGEOCHEMICAL INDICATOR CRITERIA

Sampling Protocol

Before an effective method for the evaluation of wetland biogeochemical characteristics can be established, one must identify the portion of the wetland that: (i) responds rapidly, accurately representing the impact of external loading; and, (iii) provides early warning signals of declining ecosystem health. Changes in plant community or macrofauna structures are often slow and the system may be severely degraded by the time these visual changes are observed. Concentrations of water column nutrients are often used in other water body types and are useful to determine the downstream effects of impacted wetlands. However, one prominent feature of wetland

ecosystems is that water levels often fluctuate and some wetlands have little or no period of inundation. In addition, due to the nature of hydrologic inputs, nutrient concentrations in the water column of wetlands can change rapidly and can be highly variable. This makes sampling of water column indicators somewhat unpredictable and requires more intensive sampling to quantify indicator values. Therefore, water column indices are useful to evaluate input and outflow conditions from a wetland, but do not provide the best indicator of overall nutrient condition within a wetland. However, water column nutrients are in direct contact with the microbial communities associated with periphyton, plant detritus,

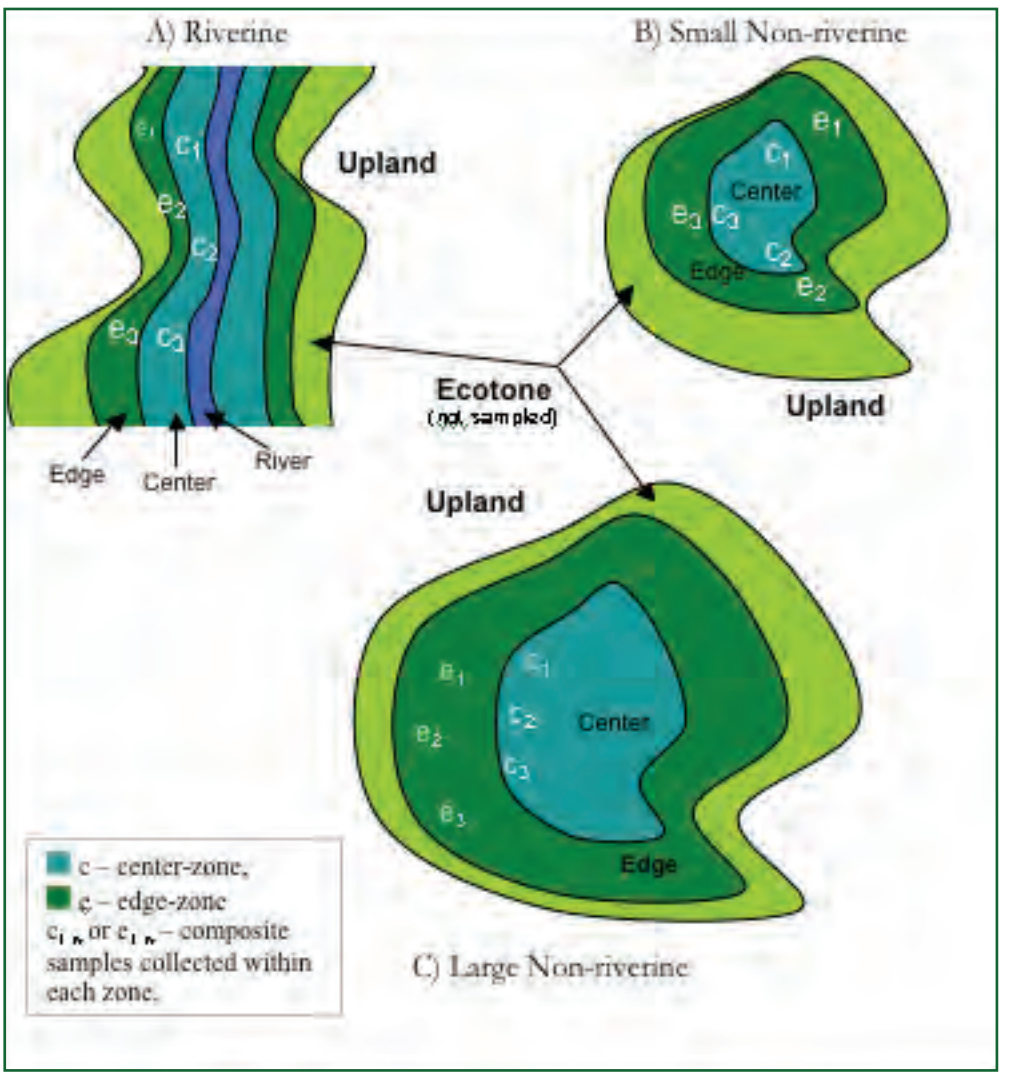

FIGURE 8: FIGURE 8. AN EXAMPLE OF THE SAMPLING SCHEME USED TO PARTIALLY QUANTIFY WITHIN WETLAND BIOGEOCHEMICAL GRADIENTS WHILE MINIMIZING WITHIN ZONE VARIABILITY AND NUMBER OF SAMPLES FOR ANALYSIS.

and surficial soils. Thus, changes in microbial community composition and activity, as well as the composition of plant detritus and soils, provide an indication of recent impact from added nutrients.

Plant detritus and soil components function as major storages of essential nutrients and serve as integrators of nutrient impacts. This integration is mediated in two ways: (i) direct assimilation of nutrients by microbes and algae colonizing detrital plant tissues in the water column, and (ii) assimilation of nutrients by plant communities and deposition of enriched detritus on the soil surface. . It should be recognized that microbes are dependent on organic substrates provided by macrophytes, while macrophytes are dependent on microbes to transform organic forms of nutrients in more bioavailable forms. This mutual dependency between microbes and macrophytes is one of the key regulators of biogeochemical processes in wetlands. Therefore, biogeochemical processes and associated microbial communities that respond rapidly to nutrient loading and related physico-chemical properties of soil, detritus, and water column, yet integrate the high variability of nutrient loading often associated with pulsed runoff events, can function as useful indicators to determine nutrient impacts.

Nutrient inputs to wetland can occur at various points from point and non-point sources. The effects of nutrient loading are usually patterned with the impacted zone adjacent to inflow points and the unimpacted zones in the interior of a wetland. Thus, monitoring stations should be located in both impacted and unimpacted zones to quantify nutrient loads accurately. Biogeochemical indicator selection and evaluation requires systematic steps before incorporation into a routine monitoring program (Figure 7). *Module 4: Study Design for Monitoring Wetlands.* provides a thorough discussion on study design and sampling protocols depending on wetland class and hydrology.

One example of a sampling design used to survey wetlands throughout the southeastern United States implemented a two zone composite sampling scheme. This technique allows for limited evaluation of wetland biogeochemical gradients due to source of nutrient inputs and hydrologic effects. It minimizes variability as well as the total number of samples to be analyzed. This sampling scheme can be applied to riparian as well as non-riparian wetlands. In both wetland types, the process begins with a visual survey of the wetland upon arrival and then divides it into two general zones, referred to as the center-zone “c” and the edge-zone “e” (Figure 8). In riverine systems, the center-zone is adjacent to the stream, but landward of any natural levees that may have formed. The edge-zone of riverine wetlands is located parallel to the upland, approximately one-third the distance between upland and stream. In non-riverine wetlands, a similar zone criteria can be applied where the center third of the wetland is designated as the center-zone and the driest third of the wetland is designated the edge-zone.

Subsampling within the center and edge zones varied slightly depending on wetland size but generally, consisted of a triplicate composite sample of soil, litter and water at each sampling station. In riverine wetlands and large (> 10 ha) non-riverine wetlands, samples were collected along one side of the wetland by collecting three subsamples

25 paces apart along a transect parallel to the wetland topographic contour. In small (<10 ha) non-riverine wetlands, samples were collected within edge and center zones at three equidistant points around the wetland.

Using this method, samples at each wetland can be collected in approximately one hour. One sample for each zone and strata is analyzed and used to characterize the wetland. Although information about within-zone variability is not retained due to compositing, greater confidence in the true central tendency of values is provided without having to run three times as many samples in the laboratory. When conducting a broad baseline survey for reference or monitoring purposes this method may provide a suitable compromise between information gained and available resources.

Water Sampling

Water quality monitoring protocols to assess trophic conditions for natural wetlands have not been established. However, wetlands used for treating wastewaters are heavily monitored, and the protocols required to monitor these systems are well established. The objective of monitoring wastewater treatment wetlands is to evaluate the nutrient removal efficiency. These wetlands are typically monitored at the inflow and outflow points for various water quality parameters. Similarly, water-sampling stations in natural wetlands should be established to capture the spatial heterogeneity caused by nutrient inputs, vegetation and periphyton communities. At the minimum, water samples should be obtained at inflow and outflow points and selected stations within wetlands. In addition, the sampling frequency should capture temporal variability within parameters being monitored. Rainfall and hydraulic loading affect water depth and concentration of various water column constituents. It also influences biotic communities. Thus, it is critical to measure water depth and stage in conjunction with selected water quality parameters at all water-sampling stations. Water samples should be collected using the protocols approved by APHA (1992) and USEPA (1993).

Soil Sampling

Wetlands exhibit a high degree of spatial heterogeneity in chemical composition of detrital and soil layers. Areas impacted by nutrients may exhibit a high degree of variability as compared to unimpacted sites within the same wetland. Thus, sampling protocols should capture this spatial variability. Developing and monitoring successful nutrient criteria management programs require sampling protocols that to capture spatial and temporal patterns.

Soil samples are usually obtained using either a grab sampling approach or collection of intact soil cores. Grab sampling is not suitable for characterizing soils, because it does not provide any indication of soil bulk density. Grab samples obtained from a constant depth (example: 0–5 cm or 0–10 cm) can be used to determine the nutrient enrichment in surface soils. However, comparison among different soils types is difficult, if intact soil cores are not obtained to determine nutrient concentrations and bulk density. For example, nutrient concentrations (expressed per unit dry weight of soil) in wetlands with light soils (such as organic soils) will be high in nutrients as

TABLE 1: POTENTIAL WATER AND SOIL QUALITY INDICATORS FOR ASSESSING NUTRIENT IMPACTS IN WETLANDS. (*DENOTES MINIMUM DATA REQUIRED FOR EACH SITE).

Level I Indicators -Water Column	Level II Indicators -Water Column
Color*	Nitrogen (NH_4-N, NO_3 + NO_2-N)
Temperature*	– Phosphorus (TDP, and DRP)
Water depth*	Total and dissolved metals (site specific situation)
Salinity/Conductivity*	Total and dissolved organic C (TOC and DOC)
Turbidity*	Total and dissolved organic N (TON and DON)
Total suspended solids*	Elemental ratios (Si:C:N:P)
Dissolved oxygen	Enzyme assays
pH and alkalinity*	– Heterotrophic respiration
Nitrogen (TKN)*	– UV absorbence
Phosphorus (TP)*	Biological N_2 fixation
	Periphyton community composition
Level I Indicators – Detritus/Soil	**Level II Indicators – Detritus/Soil**
Soil bulk density*	
Soil pH and Eh*	Enzyme assays
Cation exchange capacity	Organic matter/soil accretion rates
Total N, and P*	Stable isotope ratios
Organic matter content*	Soil-water nutrient exchange rates
Total carbon, and labile carbon	Cotton-strip assay
Particle size distribution	Detrital decomposition –litter bag
C:N:P ratios*	Microbial activity- Respiration
Extractable nutrients*	Microbial activity- Methanogenesis
Sediment oxygen demand	Microbial biomass C, N, and P
Acid volatile sulfides	– Organic N and P mineralization
Oxalate extractable metals	Nitrification
	Denitrification
	Sulfate reduction
	Substrate induced respiration
	Arginine mineralization
	Phosphorus sorption/desorption;
	Partition coefficients for P
	– P Adsorption maximum
	Degree of P saturation
	Soil mineralogical composition
	Microbial diversity

compared to wetlands with heavy soils (such as mineral soils), even though both wetlands may have similar impacts. This problem can be corrected by expressing soil nutrient concentrations per unit volume of soil, which requires the measurement of soil bulk density.

Since intact soil cores are typically used to characterize wetland soils, caution must be used to ensure that the soil is minimally disturbed when obtaining the core samples. Several approaches have been used to obtain undisturbed soil cores from wetlands. These include the use of PVC, acrylic, and aluminum tubing as coring devices. Core diameter is critical to avoid compaction. Coring tubes with diameter of <10 cm cause considerably more soil compaction than 12–15 cm tube diameters. Standard coring probes used in upland soils are not suitable for wetlands, because of saturated soil conditions and low bulk densities. Typically, organic-rich wetland soils have bulk densities in the range of 0.1–0.3 g cm^{-3} (dry weight). Nutrient concentrations expressed both on mass and volume basis should be used in evaluating the degree of impacts. However, bulk density measurements are often less precise and potentially may add an additional source of variation.

Intact soil cores with little or no detectable soil compaction can be obtained using an aluminum cylinder (15 cm diameter), with sharpened lower edge, that can be twisted through the fibrous marsh soils to a depth of 60 cm. The top of the cylinder is sealed with a PVC cap or a stopper to provide suction, and the bottom of the cylinder is sealed with a rubber stopper. The intact cores are then removed from the soil and sectioned into desired depth increments. The surface detritus is removed from the soil and saved for chemical analysis. Typically, soil cores are sectioned into 0–10, 10–30, and 30–60 cm for routine characterization. Selection of depth increments should be based on site-specific conditions and soil profile characterization. The approach described above has been used in several studies by researchers at the University of Florida and Louisiana State University. For routine monitoring of soil properties, typical root zone depth (0–10 and 10–30 cm) may adequately characterize the system.

Biogeochemical Indicators

Several biogeochemical indicators can be used as response variables to evaluate nutrient impacts in the soil and water of a wetland. Level I soil and water quality indicators are relatively easy to measure, and many are now routinely used either in monitoring aquatic systems, such as streams, rivers, lakes, and estuaries or terrestrial ecosystems. Level II indicators are relatively complex measurements, although many of these indicators are now measured in wetland systems. Level II indicators provide a better understanding of the influence of nutrient enrichment on soil processes and its ultimate effect on ecosystems function. However, both processes and indicators are regulated by various controls including nutrient loads, hydrology, fire,, and spatial/ temporal variability. Many of the Level I water and soil quality indicators can be used as independent response variables that may influence the biogeochemical processes functioning in the soil and water of a wetland. As the dependent variable, biogeochemical processes can be used as key response variables affected by nutrient loading. A list of Level I and II indicators are shown in Table 1.

Water Quality Indicators

Eutrophication, as defined, refers to an overabundance of nutrients (nitrogen and/or phosphorus) in a wetland ecosystem that produces adverse water quality impacts. In addition to nutrients, anthropogenic loads of organic matter, suspended solids, trace metals, and pesticides can also impair water quality and associated biotic communities. A number of physical, chemical, and biological water quality parameters are now used as indicators of water-body impairment. Water quality variables should be evaluated critically to obtain the most cost-effective information required to assess wetland impairment. Specifically, water quality monitoring should determine the range of values that would significantly impair the ecological integrity of wetland. The following list of variables may help address the questions related to ecological impacts on wetlands. The significance of these variables is clearly established for streams, lakes, and estuaries. Once the data are obtained, the relationships between water quality variable and response variables should be established. For example, the relationship between nutrient concentrations and response variables such as algae, vegetation, and benthic invertebrates are useful when evaluating impacts on wetlands. However, sampling of the water column is restricted to periods when the wetland is flooded. Although, the usefulness of water quality data is restricted to certain time of the year, it may provide useful information regarding short-term temporal effects on wetland biota resulting from nutrient loading. Detailed methodologies for determination of water quality parameters can be obtained from following sources: APHA (1992); Clesceri, et al., (1998), and Wetzel and Likens (1990).

TEMPERATURE, DISSOLVED OXYGEN AND PH

Water temperature controls many of the microbially mediated biogeochemical reactions in the water column. Variations in temperatures are reflected in the ranges of values for various water quality parameters and in the productivity of periphyton and vegetation. The effects of temperature on biogeochemical processes are well documented in the literature, with many of the reactions responding to temporal patterns in water temperature. In addition to distinct seasonal patterns, these temporal patterns in temperature can be observed within a diurnal cycle. However, amplitude of the daily water temperature swing depends on local climate and wetland type. Variations in temperature can be much higher in marshes with emergent vegetation, as compared to forested wetlands. Water temperature can be easily monitored with very inexpensive instruments.

Concentration of dissolved oxygen (DO) in the water column readily responds to anthropogenic impacts. Highly degraded wetlands may have wide shifts in DO concentrations. For example, wetlands receiving waters containing carbonaceous and nitrogenous oxygen demand can exhibit oxygen depletion in the water column. Oxygen production by algae can increase daytime DO concentrations and may result in low DO concentrations during the night. Among the Level I indicators, DO is and easily measurable, and commonly used by agencies involved in managing aquatic ecosystems. Oxygen is consumed during biological and chemical processes functioning in the water column. Plant, animal, and microorganisms consume oxygen during respiration. Similarly, nitrification (oxidation of ammonium to

nitrate and nitrite N) and oxidation of reduced substances such as sulfides, methane, and reduced iron and manganese consume oxygen.

Excessive oxygen production in the water column leading to super-saturation levels during daylight periods is often an indicator of nutrient enrichment in open canopy wetlands. At night, hypoxia can result from increases in detrital production, heterotrophic respiration, and release of reduced compounds at the same location. Oxygen is measured easily using commercially available oxygen probes. Single point measurements of oxygen may not be meaningful and may have very little value in evaluating impacts on wetlands. However, monitoring of diurnal fluctuations in oxygen levels continuously is more meaningful and can help explain the fate of nutrients in the water column.

The pH of the water column affects many biogeochemical processes. Water column pH can be highly variable depending on wetland type. Typically, the pH of water in many ombrotrophic wetlands (wetlands that predominantly receive hydrologic inputs from rainfall) is acidic, while pH of eutrophic wetlands is much closer to neutral. Photosynthetic activity of algae and submersed aquatic vegetation influences water column pH in poorly buffered waters. Photosynthesis results in depletion of CO_2 in the water column, shifting the carbon dioxide – bicarbonate – carbonate equilibria. However, during the night, high rates of respiration increase the production of protons, thus resulting in decrease in pH of the water column. Similar to oxygen monitoring, single point measurements of pH may not be useful in evaluating impacts on wetlands. Thus, continuous recording probes are useful in monitoring pH in the water column.

SUSPENDED SOLIDS, TURBIDITY, AND COLOR

Wetlands are effective when removing suspended solids and turbidity from inflow waters, due to lower water velocities and vegetation, which promote filtration and rapid physical settling of solids. Settling of suspended solids occurs in areas closest to the inflow point in wetlands. Many pollutants including nutrients, metals, and toxic organics are associated with suspended solids. Suspended solids often retain contaminants and can therefore maintain low concentrations of dissolved contaminants in the water column. Sampling the suspended solids in the water column within the interior marsh is often challenging, because of disturbance associated with acquiring the sample in a shallow water column and solids production within the wetland. Internal generation of solids occurs through fragmentation of detritus and litter, algal cells, and bioturbation by benthic invertebrates further contributing to the concentration of total suspended solids (TSS) in a wetland. Total suspended solids are easily measured. Water samples are acquired, filtered and dried, then weighed to determine the concentration of suspended solids (APHA 1992). In situ probes for measuring TSS are also available.

The primary causes of turbidity in water are suspended solids and color. Turbidity is measured using a turbidimeter that consists of a nephelometer, light source, and photodetector (for details see APHA 1992, EPA, 1993). The turbidity standard unit is expressed as NTU (nephelometric turbidity unit). The following relationship between turbidity and total suspended solids (has been reported by Kadlec and Knight (1995):

$$NTU = 0.83\ TSS; \quad R^2 = 0.77$$

Color is a qualitative water quality parameter. Color in surface waters of wetlands may be derived from decomposition of organic matter, plankton and phytomass, and naturally occurring metallic cations. Presence of turbidity in the water interferes with the measurement of color, thus some level of pretreatment is necessary to remove suspended solids. The color of surface waters is measured by both visual and spectroscopic methods (APHA 1992).

HARDNESS

Total hardness refers to the sum concentration of divalent cations such calcium and magnesium, both expressed as $CaCO_3$, in milligrams per liter. Surface water with low hardness is referred to as soft water, containing low concentration of calcium and magnesium. For example, rainwater is considered as soft water with calcium concentrations in the range of 0.1 to 10 mg/L and magnesium concentrations of 0.1 mg/L and a hardness value of <30 mg/L as $CaCO_3$. Many ombrotrophic wetlands and bogs typically have low base cations and can be grouped as soft water wetlands. These wetlands are typically acidic in nature. Methods to determine the hardness of water are described in APHA (1992). Hardness of water is calculated as follows:

Hardness, mg equivalent $CaCO_3$/L =2.497 [Ca, mg/L] + 4.118 [Mg, mg/L].

CONDUCTIVITY

Conductivity (also referred to as specific conductance or electrical conductivity) refers to the activity of total dissolved solids and their ability to conduct electrical current. Surface waters with a high concentration of inorganic compounds provide high conductivity. Conductance is defined as the reciprocal of resistance and is expressed as µmhos/cm. In the international system of units (SI) conductivity is reported as millisiemens per cm (mS/cm); 1 mS/cm = 10 µmhos/cm (APHA 1992). The conductivity of most natural freshwaters is in the range of 1 to 30 mS/cm, while conductivity values may reach levels >6,000 mS/cm in depressional salt lakes. The conductivity of surface water in deepwater swamps is in the range of 6–55 mS/cm (Mitsch and Gosselink 1993). Conductivity is usually measured using in situ probes (APHA 2000); additional information about conductivity and its measurement are found in APHA (2000).

SALINITY

Salinity is a measure of total dissolved ion concentration of water and is used as a reference to estuarine and marine environment including salt marshes, mangrove wetlands, and estuaries. Salinity is reported as parts per thousand (ppt) or one gram of salt in one kg of water (APHA 1992). Salinity is usually measured using an algorithm based on temperature and electrical conductivity, density, or light refraction (APHA 2000).

NITROGEN [TKN, NH_4-N, AND $NO^3 + NO_2$ –N]

Nitrogen (N) is present in organic and inorganic forms in surface waters. Methodologies to monitor surface waters are well developed for other ecosystems and can be readily adopted for wetlands. The most commonly monitored N species are total Kjeldahl nitrogen (TKN), ammonium N (NH_4–N), and

nitrate plus nitrite N ($NO_3 + NO_2$–N) (APHA 1992; EPA 1993). The TKN analysis includes both organic and ammonium N, but does not include nitrate plus nitrite N. Organic N is determined as the difference between TKN and NH_4-N. Nitrogen concentration in the water column is typically expressed as mg N/L.

Wetlands vary considerably in their capacity to process and assimilate N. For example, wetlands are very effective in removing nitrate N through denitrification process, but may not be as effective in removing ammonium N and organic N. Internally wetlands produce soluble organic N through decomposition of organic matter. Nitrogen removal efficiency is often determined by the hydraulic retention time, wetland type (including variations in vegetation and soil type), and climate. Thus, the establishment of critical range values for N needs to include the variability among wetland community types within a given region.

PHOSPHORUS [TP, TDP, AND DRP]

Phosphorus (P) entering a wetland is typically present in both organic and inorganic forms. The relative proportion of each form depends upon soil, vegetation and land use characteristics of the drainage basin. To trace the transport and transformations of P, it is convenient to classify P entering into these systems as: (i) dissolved inorganic P (DIP); (ii) dissolved organic P (DOP); (iii) particulate inorganic P (PIP); and, (iv) particulate organic P (POP). The particulate and soluble organic fractions may be further separated into labile and refractory components. Dissolved inorganic P is considered bioavailable, whereas organic and particulate P forms generally must undergo transformations to inorganic forms before being considered bioavailable. Phosphorus retention in wetlands is regulated by many factors including vegetation, periphyton and plankton, plant litter and detrital accumulation, soil physico-chemical properties water flow velocity, water depth, hydraulic retention time, length to width ratio of the wetland, P loading, and hydrologic fluctuations. When evaluating wetlands for P assimilation and establishment of critical ranges, it is necessary to consider: (i) short-term storage mediated by assimilation into vegetation, periphyton and incorporation into detrital tissue; and, (ii) long-term storage mediated by soil assimilation, and accretion of organic matter.

Phosphorus concentration in the water column is typically expressed as µg/L or mg/L. Methodologies to monitor P in surface waters are well developed for other ecosystems and can be readily adopted for wetlands. The most commonly monitored for P species are total P (TP), dissolved reactive P (DRP), and total dissolved P (TDP) (APHA 1992; EPA 1993).

ALKALINITY

Alkalinity of water refers to its acid neutralizing capacity and is primarily a function of carbonate, bicarbonate, and hydroxide levels. It is expressed as mg $CaCO_3$/L. A number of wet chemistry methods are available to determine alkalinity of surface waters in wetlands (APHA 1992).

TOTAL, DISSOLVED AND PARTICULATE ORGANIC CARBON

Total organic carbon (TOC) in surface waters includes: dissolved organic carbon (DOC)—the fraction of TOC that passes through a 0.45 um pore diameter filter, and particulate organic carbon (POC)—the fraction of TOC that is retained on the 0.45 um diameter filter. Since some of the organic matter in surface water can be oxidized or utilized by microbes, simple methods such as biochemical oxygen demand (BOD) and chemical oxygen demand (COD) are often used as indicators of organic carbon (APHA 1992). However, total organic carbon may contain biodegradable and recalcitrant organic matter. Thus, direct measurement of TOC may be more useful in determining the water column organic carbon content. A number of methods such as direction combustion at high temperature or combustion using infrared method, or persulfate-ultraviolet oxidation method are used to determine TOC in surface waters (APHA 1992). The TOC concentrations can also be related to the turbidity or color of the surface waters. Dissolved organic C, TOC, and POC concentrations are expressed as mg/L.

SULFATE AND SULFIDE

In the absence of oxygen, nitrate, and oxidized forms of iron and manganese, obligate anaerobic bacteria can use sulfate as their electron acceptor during respiration, while using organic matter as their energy source. Under most wetland conditions, sulfates in the water column are stable. However, sulfates can diffuse from water column to underlying soil, where they can be reduced to sulfides. In many freshwater wetlands, sulfate concentrations are low, so monitoring them may not be critical for water quality. However, recent linkages between sulfate reduction and mercury methylation in wetlands suggest that monitoring sulfates for site-specific conditions may be essential. Accumulation of sulfides in the soil may have positive effects on availability of metals, as sulfides can precipitate metals into insoluble forms. This process is very important in coastal wetlands where sulfate concentrations are several fold higher than freshwater wetlands.

Sulfate concentrations are expressed in mg/L. Sulfate in surface waters can be analyzed using ion chromatographic method or gravimetric method (APHA 1992). Sulfides can be measured using a sulfide electrode or by wet chemistry methods (APHA 1992).

METALS

Monitoring wetlands for metals can be important under site-specific conditions. Metals can be determined using several methods including atomic absorption, inductively coupled plasma (ICP), or using wet chemistry methods (APHA 1992). These methods are widely used by both governmental and commercial laboratories. Metal concentrations in the water column are expressed as mg/L or µg/L.

Dissolved metals are those constituents (metals) of a sample that pass through 0.45 um filter and are preserved under acid conditions (pH <2). Acid extractable metals represent the concentration of metals in the sample after treatment of unfiltered sample with hot dilute mineral acid. Total metals are those determined in unfiltered sample after digestion with acids. Several digestion methods are routinely used (APHA 1992).

Soil Quality Indicators

Soil quality may be viewed as the capacity of the soil to function within ecosystem boundaries and to sustain biological productivity, maintain environmental quality, and promote plant and animal health. Soil includes both native soil and detrital/litter components which potentially convert into soil organic matter. A number of soil and detrital physical, chemical, and microbial parameters can be used as potential indicators of quality. We will use soil indicators that directly or indirectly reflect quality, and can be linked with environmental or anthropogenic factors. For example, dissolved constituents and those bound on soils contribute significantly to surface water quality. Chemical and microbial indicators can also provide important links to soil quality. This class of ecological indicators is associated with biogeochemical cycling, the turnover and storage of nutrients and other elements in biotic and abiotic compartments of the ecosystem. Therefore, it is related to biological productivity in the soil as well as in the floral and faunal components of the ecosystem. Recent studies in terrestrial and wetland ecosystems have demonstrated the potential of using microbial communities and process measurements as sensitive indicators of environmental perturbations in soils and sediments (Torstensson, et al.1998), including heavy metals (Frostegard, et al.1993), physical disturbance (Findlay, et al.1990), and nutrient impacts (DeBusk and Reddy 1998; Reddy, et al. 1999).

Decomposition of organic matter is the primary ecological role of heterotrophic microflora in soils. It provides for mineralization of potentially growth-limiting nutrients and formation of recalcitrant organic compounds (e.g. humus) that contribute to the chemical stability of the system (Swift 1982; Jorgensen, et al. 1999; Middelboe, et al. 1998). Soil microbes may also exert a significant influence on ecosystem energy flow in the form of feedback, since the mineralization of organically bound nutrients is a regulator of nutrient availability for both primary production and decomposition. Therefore, most of the net ecosystem production passes through the microbial compartment at least once and typically several times, although microbial biomass comprises only a small fraction of the sediment organic matter (Jorgensen, et al. 1999; Ruttenberg and Goni 1997; Seitzinger and Sanders 1997). Many of the process level parameters are tedious to measure and require specialized training and equipment; thus, they may not be suitable for routine monitoring in wetlands.

Monitoring soil properties may provide long-term integrated effects of nutrient impacts on wetlands, but may not be suitable to determine short-term temporal changes in the system. Chemical composition of soils and detrital components of wetlands can be easily monitored using approaches similar to that used in soil testing for plant nutrition in agricultural ecosystems. However, some modifications are necessary to adopt these methods to wetland environment. Detailed methodologies for determination of soil quality parameters can be obtained from following sources: Dane and Topp, (2002), Klute (1986), Sparks, et al. (1996), Weaver, et al. (1994).

SOIL BULK DENSITY

Soil bulk density refers to the ratio of the mass of dry solids to the bulk volume of soil (Blake and Hartge 1996). The bulk volume

includes the volume of solids, water, and pore space. The mass of solids is determined after drying the soil at 105°C and the bulk density is calculated as follows:

Bulk density (dry) (g/cm^3) = (mass dry weight, grams)/volume (cm^3)

Bulk density of wetland organic soils ranges from 0.1 to 0.4 g/cm^3, while for mineral wetland soils values can be in the range of 1 to 1.5 g/cm^3. This is a useful parameter, when concentration of nutrients is expressed on a volume basis rather than mass basis. For example, concentration of nutrients in organic wetland soils can be high when expressed as mg/kg of dry soil (µg/g of dry soil), as compared to mineral wetland soils. However, the difference in concentration may not be as high when expressed on a volume basis. Nutrient availability to plants is regulated by the total amount of nutrients available in soil volume. Bulk density is determined on intact soil cores obtained at known depth and volume. Volume is determined from core diameter and depth, while the dry weight of the soil per core volume is determined as described above.

SOIL PARTICLE SIZE DISTRIBUTION

Particle size analysis (PSA) is a measurement of size distribution of individual particles in a soil sample (Gee and Bauder 1986). Particle size analysis is only applicable to mineral wetland soils. Soil particles cover a wide range including clay (<0.002 mm), silt (0.002 to 0.02 mm), fine sand (0.02 to 0.25 mm), coarse sand (0.25 to 2 mm), and gravel (2 to 80 mm). Particle size analysis is often used to determine soil texture, which is based on various combinations of sand, silt , and clay separates that make up the particle size distribution of a soil sample (Gee and Bauder 1986). Particle size distribution also correlates to the phosphorus and metal retention capacity of soils. Typically the larger the clay and silt fraction of a sample the greater capacity the soil has for P and metals retention; however, this is also highly dependant upon the mineralogy of these smaller size fractions. Several simple methods are described by Gee and Bauder (1986) for PSA of mineral upland soils, which can be readily applied to wetland soils.

REDOX POTENTIAL

Oxidation and reduction reactions regulate many of the biogeochemical reactions in wetlands. Electrons are essential to many biogeochemical reactions. Oxidation, the loss of electrons, couples with reduction in the gain of electrons. The Eh of soil is determined by the concentration of oxidants and reductants. Oxidants include oxygen, nitrate, nitrite, manganic manganese, ferric iron, sulfate and CO_2, while reductants include various organic substrates and reduced inorganic compounds. From the ecosystem point of view, photosynthesis and respiration are two examples of reduction and oxidation reactions, which regulate energy flow and many biogeochemical reactions. For example, during photosynthesis, CO_2 is reduced to carbohydrate, and during respiration the reduced organic compounds are oxidized to CO_2. Photosynthesis provides an organic matter source to the soil through plant productivity and detrital accumulation. Compared to upland soils, one of the most striking characteristics of a wetland soil is its low Eh, which is a measure of elec-

tron activity or potential in the soil. Redox potential of soil is measured using a platinum electrode with a standard calomel reference.

Redox potential (Eh) measurements have been widely used to characterize the propensity of wetland soils to oxidize or reduce substances. The value of these measurements depends on their interpretation with due recognition to its theoretical and practical limitations. Redox potential is the best available simple indicator of the oxidation-reduction status of soil, for the following reasons: (i) the range of Eh values in wetland soil is much wider, approximately 1000 mV, than the 300 mV range of Eh values in drained soils; (ii) the higher concentrations of reductants that contributes to the mixed potential in wetland soils result in better poising and better reproducibility; (iii) O_2 is easily reduced and, therefore, usually present at negligible levels in wetland soils. Methods used to determine O_2 status of drained soils cannot be used in wetland soils; and, direct measurement of reducing compounds (i.e., the presence of ferrous iron, manganese, or sulfides or methane) is cumbersome and difficult to perform on a routine basis. Thus, the redox potential remains a generally applicable, reasonably convenient way to identify the presence and intensity of reduction in wetland soils.

Redox potential is significantly affected by the oxygen content of the soil. In systems where aerobic organisms function, the Eh range is very narrow, ranging from approximately +700 mV down to about +300 mV. Below values of 300 mV, facultative anaerobes function down to about 0 mV. Below this range, obligate anaerobes function. In wetland (waterlogged or flooded) soils, Eh can be anywhere along the entire scale. Where oxygen is present in wetland soil the Eh can be as high as in a drained soil, but where oxygen is not present Eh can be low (-250 to -300 mV).

Redox potentials in soils are measured in: (i) soil porewater; (ii) soil slurry; (iii) intact soil cores; or, (iv) in situ under field condition. Redox potential is measured using a platinum electrode (custom made or purchased commercially) and reference electrode (usually calomel electrode), both inserted in the soil or soil slurry. Both electrodes are connected to a pH/millivolt (mV) meter. Readings stabilize within minutes; however, it is recommended that electrodes be left for at least 24 hours before measurements are taken in the field. A longer equilibration period is necessary because of the heterogeneous nature of soil systems. The reference electrode does not have to be at the same depth as platinum electrode, as long as there is enough soil moisture to insure a good electrical contact. However, the distance between the platinum electrode and the reference electrode can affect the electrical resistance of the measuring circuit. This may not be a serious problem in saturated soils. For details on methodologies, see Patrick et al., 1996.

There are several commercially available electrodes designed to measure Eh, pH and specific ion activities. This section will present simple methods to construct redox electrodes for use in the laboratory and under field conditions. Many commercially available electrodes are bulky and are not suitable for use under field conditions. Methods associated with the construction of redox electrodes were developed for the past three decades in our laboratories.

Field electrodes are typically made with platinum wire with a diameter of 1.024 mm cut into 1 to 1.5 cm segments. Smaller gauge platinum is not recommended because of poor reproducibility of potentials. These segments are soaked in a concentrated acid (1:1 HNO_3 and HCl mixture) for several hours (< –4 hours) to remove surface contamination of metals and other impurities, followed by thorough washing in deionized distilled water. The platinum segments are soldered or fused to a copper lead (1.628–2.053 mm diameter) of desired length. Waterproof epoxy glue is used to cover the joint between copper and platinum. The copper wire is inserted into a heat shrinking tube to proved insulation. The platinum tip end of the copper wire is sealed with epoxy, while at the other end, about 3 cm of copper wire is exposed. This end is connected to an insulated cable connected to a pH/volt meter. This type of electrode can be left installed in the field for a period of about one year for seasonal measurements. However, it is recommended that the electrodes are periodically checked and reinstalled in the field (at least once every three months).

Redox potentials or Eh values are typically expressed in reference to a standard hydrogen electrode (SHE). Using a SHE is not practical; therefore, alternate reference electrodes are used. Reference electrodes (calomel electrodes) are available commercially through scientific catalogs or can be prepared in the laboratory as follows. Calomel electrodes are checked by substituting a standard calomel electrode for the platinum electrode and verifying a zero potential difference between the two half-cells. Commercially available reference electrodes are convenient for use under field conditions. To convert electrode potential meter readings to Eh values using calomel reference electrodes, the meter readings are adjusted by adding +245 mV to the readings. For example, if the electrode potential meter using calomel electrode reads -100 mV, then the actual corrected Eh values will be: (-100 mV + 245 mV) = 145 mV. If silver/silver-chloride reference electrodes were used, then +199 mV is the correction factor. Note that these correction factors are temperature dependent. However, changes in potential due to temperature are usually small compared to the variability in electrode potential measurements made in the field. Thus, correcting for temperature effects on Eh values is not critical.

SOIL PH

The pH of wetland soils and water varies over a wide range. Organic wetland soils are often acidic, and mineral wetland soils are frequently neutral or alkaline. Flooding a soil results in the consumption of electrons and protons. In general, flooding acidic soils increases the pH, while flooding alkaline soils decreases pH (Mitsch and Gosselink 1993). The increase in pH of flooded soils is largely due to the reduction of iron and manganese oxides. However, the initial increase in pH can also occur due to rapid decomposition of soil organic matter and accumulation of CO_2. The decrease in pH that generally occurs when alkaline soils are flooded results from the buildup of CO_2 and carbonic acid. Additionally, the pH of alkaline soils is highly sensitive to changes in the partial pressure of CO_2. Carbonates of iron and manganese can also buffer the pH of soil to neutrality.

Soil pH is measured using commercially available combination electrodes on soil slurries. If air dry or moist soil is used, a 1:1 soil

to water ratio should be used. For details on methodology, the reader is referred to Thomas (1996).

LOSS ON IGNITION OR SOIL ORGANIC MATTER

Wetlands are characterized by the accumulation of organic matter resulting from high productivity and slow rate of decomposition. In addition to providing nutrient storage and supply, organic matter also increases the cation exchange capacity of soils, increases the adsorption or deactivation of organic chemicals and trace metals, and improves overall soil structure. Loss on ignition (LOI) is a measurement of the organic matter content of soil. A number of methods are now routinely used to estimate organic matter content of the soil and expressed as total organic carbon or LOI. Significant relationships have been reported between total carbon and organic matter content or LOI. The ratio of organic matter content to total carbon ranges from 1.8 to 2.2, while the ratio of LOI to total carbon was found to be 2.6. The ratio of organic matter to LOI was reported to be in the range of 0.57 to 1.13 for a wide range of mineral upland soils (Nelson and Sommers 1996).

TOTAL NITROGEN AND PHOSPHORUS

Total Kjeldahl nitrogen (TKN) in soils and detritus/litter material is determined by converting organic forms of N to NH_4-N by digestion with concentrated H_2SO_4 at temperatures of 300-350°C (Bremner 1996). The NH_4-N in digested samples is analyzed using colorimetric methods (APHA 1992). Methods of total N determination are well defined and are now routinely used by many laboratories.

Total P in soils and detritus/litter material is determined by oxidation of organic constituents and acid dissolution of minerals at temperatures of <300°C (Kuo 1996). Digested solutions are analyzed for P using colorimetric methods (APHA 1992). Methods of total P determination are well defined and are now routinely used by many laboratories.

ORGANIC MATTER AND SEDIMENT ACCRETION

Organic matter accretion refers to accumulation of detrital material derived from vegetation and algae, while sediment accretion refers to the accumulation of both organic and inorganic material. Wetlands are effective in trapping sediments. Sediment accretion can be measured by placing a suitable marker, such as feldspar, and measuring the accumulation of both organic and inorganic material over time.

Accretion rates are also determined by gamma analysis of the 661 keV photopeak for ^{137}Cs in each increment using a low-energy germanium detector (Canberra Industries, Meriden, CT). Cesium-137 (half-life 30 yr), produced from above-ground thermonuclear weapons testing, can be used as a marker to estimate recent (30–40 yr) rates of sediment deposition and nutrient accumulation (Ritchie and McHenry 1990; Craft and Richardson 1993; Reddy, et al. 1993). Atmospheric deposition of ^{137}Cs first began in 1954, with peak fallout occurring in 1964 (Ritchie and McHenry 1990). These two dates are most frequently used to measure sedimentation rates (Ritchie and McHenry 1990). However, the 1954 sediment horizon is sometimes difficult to discern because of the low ^{137}Cs activity (compared to the 1964 peak), bioturbation and diffusion of

^{137}Cs (Ritchie and McHenry 1990). For this reason, the 1964 peak, frequently is used as the marker layer in wetland studies (Craft and Richardson 1993a,b; Reddy et al. 1993). Thus, the maximum ^{137}Cs level in the soil profile corresponds to the time of maximum deposition of ^{137}Cs fallout, approximately 1964.

CALCULATING SEDIMENT AND NUTRIENT ACCUMULATION RATES

Nutrient (C, N, P) accumulation rates were calculated as follow.

Nutrient Accumulation Rate = BD * NC where:

BD = mean bulk density above the ^{137}Cs peak

NC = mean nutrient concentration above the ^{137}Cs peak

Sediment accumulation rates are calculated similarly by subtracting the dry mass of organic matter from the total dry mass of the soil based on the assumption that soil organic matter is 50 percent organic C.

POREWATER AND WATER EXTRACTABLE NUTRIENTS

Movement of nutrients and other contaminants between the soil and water column depends on their concentration in the soil pore water (interstitial water) and in the water column. Water content in wetland soils may vary. Water content of mineral soils is typically in the range of 30–50%, while organic soils may have up to 95% water content. Recently deposited flocculent sediments may have water content of up to 99%. Some of this water is held by soil particles through capillary forces and trapped in the crystal lattice of minerals. A major portion is present as free water. This interstitial water, called porewater, fills the space between soil particles. Sampling porewater is laborious and requires special equipment, and may require some experience and knowledge of the chemical interactions in soils. For certain chemical parameters, such as dissolved P, iron, manganese, and sulfides, it is critical that porewater extractions are performed under oxygen-free conditions. Common methods used to extract porewater include squeezing and centrifugation. Sulfide analysis must be performed on unacidified samples. In situ porewater sampling includes the use of porewater sippers or porewater equilibrators commonly known as "peepers" (Hesslein 1972). These methods may not be suitable for routine sampling of porewater due to disturbance and duration of deployment. If the goal of sampling is to determine the dissolved concentration of metals, ammonium N, and P, samples must be preserved under acid conditions (pH <2). Analysis for these dissolved constituents can be performed using standard methods as described for water quality indicators.

Under certain conditions, it may not be possible to extract porewater constituents, especially during low water-table conditions. Under these conditions, soil samples can be extracted with distilled water at a soil (dry weight) to water ratio of 1:4, after equilibration for a period of one hour on a shaker table. Soil suspensions are centrifuged and filtered through 0.45 um filter. The filtrates are acidified to pH <2 and stored at 4°C for analysis.

EXTRACTABLE PLANT AVAILABLE NUTRIENTS AND METALS

(USING STANDARD SOIL TEST PROCEDURES RELEVANT TO THE REGION)

Selected methods used to determine P and metals are described below. Many soil-testing laboratories perform this analysis on a routine basis. The following extractions can be performed on air-dried soil that has been ground to pass a 2 mm sieve.

Mehlich-I: The Mehlich-I extraction solution consists of 0.05 *M* HCl and 0.0125 *M* H_2SO_4. It is typically used in the Southeast and Mid-Atlantic regions on mineral soils with pH of <7.0. The extractant consists of dilute concentrations of strong acids. Many of the plant nutrients such as P, K, Ca, Mg, Fe, Zn, and Cu extracted with Mehlich-I methods have been calibrated for the production of crops in agricultural ecosystems. This solvent extracts some of Fe and Al-bound P, and some Ca-bound P. Soil (dry) to extraction ratio is set at 1:4 (5 g of soil plus 20 ml of extractant solution), for mineral soils. Wider ratios should be used for highly organic soils. The soil solutions are equilibrated for period of 5 minutes on a shaker table and filtered through Whatman No. 42 filter. Filtered solutions are analyzed for P and other nutrients using standard methods.

If the filtrate for a sample contains 10 mg P/L, the soil contains 40 mg P/kg, as shown below:

Mehlich-I-P = [(10 mg P/L) (0.020 L)]/0.005 kg soil = 40 mg P/kg of soil

Mehlich-III: The Mehlich-III method was developed to evaluate levels of available nutrients in soils of southeastern United States (Mehlich 1984). The Mehlich-III extraction solution consists of 0.2 *M* CH_3COOH and 0.25 *M* NH_4F, 0.013 *M* HNO_3, and 0.001 *M* EDTA. Inclusion of ammonium fluoride provides a better estimation of P availability in near-neutral and alkaline soils than acid solutions used in Mehlich-I method. The chelating agent EDTA aided in the extraction of available metals. Many of the plant nutrients (such as P, K, Ca, Mg, Fe, Zn, and Cu) extracted with Mehlich-III methods have been calibrated for the production of crops in agricultural ecosystems. Soil (dry) to extraction ratio is set at 1:8 (2.5 g of soil plus 20 ml of extractant solution), for mineral soils, while wider ratios should be used for highly organic soils. Soil solutions are equilibrated for period of 5 minutes on a shaker table and filtered through Whatman No. 42 filter. Filtered solutions are analyzed for P and other nutrients using standard methods.

Bray P-1: This method has been widely used as an index of available P in soils. The combination of dilute concentrations of a strong acid, HCl (0.025 *M*), with ammonium fluoride (NH_4F at 0.03 *M*) is designed to easily remove acid-extractable soluble P forms such as Ca-bound P, and a portion of Fe and Al-bound P. The NH_4F dissolves Fe and Al-bound P by forming complex ions with these metal ions in acid solutions. This method has been successfully used on acid soils. Soil (dry) to extraction ratio is set at 1:7 for mineral soils, while wider ratios should be used for highly organic soils. Soil solutions are equilibrated for period of 5 minutes on a shaker table and filtered through Whatman No. 42 filter. Filtered solutions are analyzed for P and other nutrients using standard methods.

Bicarbonate Extractable P: This method is suitable for calcareous soils. Soil P is extracted from the soil with 0.5 *M* $NaHCO_3$, at nearly a constant pH of 8.5. In calcareous, alkaline, or neutral soils, containing Ca-bound P, this extractant decreases the concentration of Ca in solution by causing precipitation of Ca as $CaCO_3$; and as result P concentration in soil solution increases. Soil (dry) to extraction ratio is set at 1:20 for mineral soils and 1:100 for highly organic soils. Soil solutions are equilibrated for period of 30 minutes on a shaker table and filtered through Whatman No. 42 filter. Filtered solutions are analyzed for P using standard methods.

OXALATE EXTRACTABLE ALUMINUM AND IRON

A number of researchers have shown a strong relationship between the P retention capacity of soils and the ammonium oxalate extractable Fe and Al content of the soils (Reddy, et al. 1998b). This extractant dissolves poorly crystalline and amorphous forms of Fe and Al, which are primarily found to retain P in acid mineral soils. Thus, measurement of oxalate extractable Fe and Al provides an index of P retention capacity of soils. Air dried soils are extracted with 0.1 *M* oxalic acid + 0.175 *M* ammonium oxalate (pH = 3.5) at a soil to extractant ratio of 1:50. After 4 hour equilibration on a mechanical shaker table, soil suspensions are centrifuged and filtered through 0.45 µm filter. Filtered solutions are analyzed for Fe, Al, and P using standard methods.

SOIL OXYGEN DEMAND

Soil oxygen demand (SOD) can be measured using an O_2 electrode and incubation of materials in biological oxygen demand (BOD) bottles for a 24-hour period (WBL 2001). A known amount of wet soil (5–10 g) is weighed into a 250 mL BOD bottle containing a stir bar. The bottle is filled with deionized distilled water under continuous stirring on a magnetic stirrer. The contents are stirred for a period of 15 minutes and the DO is measured. The bottle is sealed with a glass stopper and placed in the dark at 25°C for a period of 24 hours. At the end of incubation, DO is measured under continuous stirring. If the DO levels decrease by more than 50%, analysis should be repeated with smaller sample size. Soil oxygen demand is calculated as follows:

SOD (mg/kg-hour) = [**{**[**DO**, mg/L]$_{t=0}$ – [**DO**, mg/L]$_{t=24\ hours}$**}** **V**, L] / dry weight of soil , kg

Where: DO = dissolved oxygen;
V = volume of water in L, liters

LIGNIN AND CELLULOSE CONTENT

(LIGNO-CELLULOSE INDEX)

Decomposition rate is significantly affected by chemical composition of detrital tissue and soil organic matter. Lignin and cellulose comprise major components of organic matter, dictating the rate of decomposition and substrate quality. Cellulose is more easily biodegraded than lignin. The ligno-cellulose index [LCI = (lignin/lignin + cellulose)] has been used to characterize substrates for their decomposability. The ligno-cellulose index can be calculated from these measurements and used to characterize the decomposability of sampled soil material. For example, during decomposition, the LCI for cattails was shown to increase from 0.2 to 0.8, with low

values observed in easily decomposable live tissue and high values observed in recalcitrant litter incorporated into soil organic matter (DeBusk and Reddy 1998).

DETRITAL/LITTER DECOMPOSITION
(LITTER BAG MEASUREMENTS)

Decomposition of detrital/litter material in wetlands results in the release of nutrients into the water column. Ecologists have used simple litter-bag methods to determine the decomposition rate of plant tissue (Aber and Melillo 1980; Wieder and Lang 1982). Typically detrital/litter material is chopped into (approximately 2–5 cm in length), and known amount of fresh plant tissue is placed in litter bags (approximately 15 x 30 cm^2) constructed of fiber glass screening (1 mm mesh). The edges of litter bags are stapled at 5 cm interval, to provide relatively large openings along the periphery for macroinvertebrate access. The bags, with detrital tissue, are placed on the soil surface or in the detrital layers. The bags are randomly removed at predetermined time intervals, carefully rinsed to remove external debris, and the detrital tissue is dried at 70°C for a period 72 hours, and dry weights are recorded. Loss in dry weight of the litter provides an indication of the rate of decomposition.

COTTON-STRIP ASSAY

Soil organic matter decomposition is an important process for the autochthonous mobilization of nutrients for plant growth and controlling organic matter accumulation. The decomposition of cellulose strips has been used extensively in the literature as a surrogate for plant organic matter decomposition, providing a method for normalizing substrate quality between sites (French 1988; Harrison, et al. 1988). The cotton strip technique for quantifying cellulose decomposition is based on the loss of tensile strength of cellulose fibers, referred to as cotton tensile strength loss (CTSL), of a standardized cotton fabric comprising 97% holocellulose (Latter and Howson 1977; Latter and Harrison 1988). The cotton strip technique evaluates decomposition by measuring loss of tensile strength of the cotton fibers making up the strips. Measurements of tensile strength loss rates have been undertaken and proven successful in a wide range of wetlands (Maltby 1987; Newman, et al. 2001) and non-wetland environments (Harrison et al. 1988) throughout the world. At each location, replicate cotton strips of the standard material (Shirley Institute Test Fabric, Didsbury, England) and size (12 x 30 cm^2 or longer depending on objectives) are inserted vertically into the soil substrate with the aid of a sharpshooter shovel as described by Maltby (1987). Control strips are inserted and removed immediately. The remaining strips are exposed for usually a 10–14 day period, depending on the rate of decomposition. After retrieval, all strips are immediately washed in freshwater to remove soil and debris and washed again in deionized water. Samples are dried at room temperature and stored in plastic bags until analysis. The strips are cut into horizontal segments 3 cm wide and reduced by fraying to 2 cm segments that result in test units corresponding to depths of 0–2, 3–5, 6–8 cm, etc. Loss of tensile strength is measured from each 2 cm x 12 cm segment. Tensile strength is measured with a tensometer (Monsanto Type-W or equivalent) equipped with 7.5 cm wide jaws adjusted to 3 cm spacing. All measurements are carried

out at 18–22°C and 100% relative humidity obtained by soaking the strips in deionized water. Individual losses in tensile strength are calculated relative to the field controls obtained for each site. These data are used to calculate percentage mean loss of tensile strength for each level in the profile. This procedure is sensitive to differences in soil fertility (Maltby 1985, 1987; Mendelssohn, et al. 1999).

MICROBIAL ACTIVITIES

Microbial parameters (listed in Table 1, Level II) are now routinely measured by researchers. Examples of some of these parameters include: (i) extracellular enzyme activity (Wright and Reddy 2001a); (i) microbial biomass C, N, and P (Ivanoff, et al. 1996; White and Reddy 2000); (ii) microbial respiration and methanogenesis (Wright and Reddy 2001b); (iii) potentially mineralizable N (Wright and Reddy 2000); (iv) potentially mineralizable P; and, (v) denitrification enzyme activity (White and Reddy 1999). Details about methodologies can be obtained from the references cited.

Minimum Monitoring Requirements

Nutrient related data on water and soil quality indicators in wetlands is limited. To date, much of the data collection is at the experimental scale for site-specific conditions. Assessing wetland eutrophication requires systematic data collection at a large spatial scale, using comparable techniques. Recognize that under most conditions adequate resources might not be available to obtain detailed data even for Level I indicators. However, wetland nutrient assessment requires minimum data collection and evaluation of soil and water quality indicators. This document presents a simple, systematic approach to minimal data collection. Level I indicators required for minimum data needs are listed in Table 1.

The location of suitable field sites should be coordinated through local academic or governmental agencies to determine appropriate reference sites for sampling (see Module #4—Study Design for Monitoring Wetlands). Criteria for reference site selection should be based on areas of least cultural impact within a particular region as determined by the local knowledge source. Once identified, sites should be characterized using a standard classification scheme (Cowardin, et al. 1979) and by community characteristics. Latitude and longitude of the site should also be collected for use in relocation and cross-referencing with other geographic information system (GIS) sites.

Sampling at selected sites should consist of three composite samples collected from the water column, detritus and soil. Water samples, when available, should be collected at a mid-water depth, filtered, homogenized (with other replicates) then stored on ice or preserved until analysis. Intact soil should be collected to a depth of 10 cm below the litter/soil interface. Litter from these cores, as defined by easily distinguishable plant fragments lying on the surface of the core, should be collected, air dried, and then combined with other detritus samples from the site. The remaining upper 10 cm of soil from each core should be air dried, then combined with site replicates.

Laboratory analysis of water column composite samples should include total nitrogen and total phosphorus. Soil and detritus composite samples should be air-dried at 25 to 30°C, ground, and homogenized. Aliquots should be analyzed for organic matter content, pH (to be determined on ambient wet sample), total nitrogen, total phosphorus, extractable ammonium N (to be determined on ambient wet sample), and extractable phosphorus, iron, aluminum, calcium, and magnesium. Moisture content of air-dried detrital matter and soils should be determined after over drying samples at 70°C for 2–3 days or until constant weights are recorded. All nutrient concentrations determined on air-dried samples should be normalized on an over-dried basis.

Case Study: The Everglades

The Everglades is one of the most unique subtropical wetland ecosystems in the world. It evolved biologically from an organic matter accumulation in a low-nutrient environment sitting within a limestone depression (Davis 1943). Historically, the major source of nutrients to the Everglades has come from atmospheric deposition, with minimum secondary nutrient inputs through infrequent sheet flooding in the northern Everglades from Lake Okeechobee. Nutrient limitation, hydrology, and fire are several key factors in the establishment of the endemic Everglades flora, which has adapted to the low nutrient environment (Davis 1991). Nutrient loading to Water Conservation Areas (WCAs) of the northern Everglades has not only altered the vegetational communities, but also increased nutrient accumulation (Davis 1991; DeBusk, et al. 1994; Newman, et al. 1997). Although the effects of nutrient loading and altered hydrology on changes in plant communities are clearly evident, very limited information is available on the influence of these factors on biogeochemical processes regulating nutrient availability and cycling in impacted and unimpacted areas. Nutrient accumulation rates of 0.11–1.14 g m^{-2} yr^{-1} potassium and 5.4–24.3 g m^{-2} yr^{-1} nitrogen have been reported for the Everglades (Craft and Richardson 1993a,b; Reddy, et al. 1993). The highest accumulation rates were noted in areas closest to the source of nutrient inputs, and the lowest accumulation rates occurred in areas furthest from the input points. A brief summary of the results of various biogeochemical indicators measured along a nutrient enrichment gradient in WCA-2a of the Everglades are listed in Table 2 (Reddy, et al. 1999).

Minimum Data – Level I

- **Water column:**
  - Water depth
  - Total Nitrogen
  - Total Phosphorus
- **Litter/Detritus:**
  - Total carbon,
  - Total nitrogen
  - Total phosphorus
- **Soil:**
  - Bulk density
  - Organic matter content,
  - Total carbon,
  - Total nitrogen,
  - Total phosphorus,
  - Extractable nitrogen (2M KCl),
  - Extractable phosphorus (Mehlich – 1 and 3)
  - Extractable Fe, Al, Ca, Mg, and K

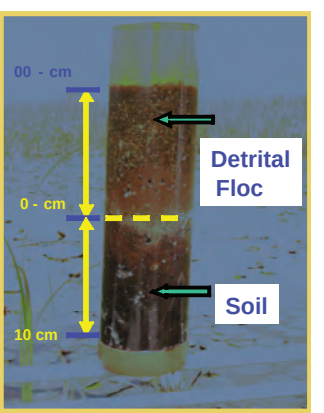

TABLE 2A: BACKGROUND CONCENTRATIONS (STANDARD ERROR) SELECTED BIOGEOCHEMICAL INDICATORS IN FLOC/DETRITAL COMPONENT OF VARIOUS HYDROLOGIC UNITS OF THE EVERGLADES. THE PMN AND PMP REFER TO POTENTIALLY MINERALIZABLE N AND P, RESPECTIVELY (WRIGHT, ET AL. 2002)

Parameter	Units	WCA -1	(SE)	WCA -2A		WCA -3A		TS	
FLOC/ DETRITUS									
Loss on Ignition	%	83	(3)	50	(3)	91	(0)	39	(3)
Extractable C	g C kg^{-1}	7	(1)	5	(0)	12	(1)	7	(1)
Extractable NH_4-N	mg N kg^{-1}	-		181	(8)	379	(40)	177	(8)
Labile P	mg P kg^{-1}	4	(4)	1	(1)	6	(1)	1	(0)
Total P	mg P kg^{-1}	227	(21)	315	(22)	540	(16)	156	(9)
Total inorganic P	mg P kg^{-1}	51	(6)	157	(11)	174	(15)	58	(4)
Total N	g kg^{-1}	35	(1)	18	(1)	45	(0)	17	(1)
Total C	g kg^{-1}	413	(9)	261	(11)	448	(3)	250	(9)
Microbial Biomass C	g C kg^{-1}	24	(14)	14	(2)	30	(5)	10	(3)
Microbial Biomass N	mg N kg^{-1}	1346	85	1294	(202)	2879	(250)	1171	(334)
Microbial Biomass P	mg P kg^{-1}	305	(65)	117	(17)	87	(22)	53	(25)
PMN	mg N kg^{-1} d^{-1}	-		88	(10)	212	(25)	49	(7)
PMP	mg P kg^{-1} d^{-1}	-		5	(2)	24	(8)	2	(0)
Soil O_2 Demand	mg kg^{-1} hr^{-1}	-		-		89	(12)	26	(3)
CH_4 Production	mg C kg^{-1} d^{-1}	-		118	(7)	564	(53)	181	(23)
Aerobic CO_2 Prod.	mg $kg^{-1}h^{-1}$	53	(1)	36	(4)	-		-	
Anaerobic CO_2 Prod.	mg $kg^{-1}h^{-1}$	36	(11)	34	(15)	-		-	

Observations made in the Everglades wetlands are applicable to nutrient limited systems. Although, the indicators presented in this report will be same for any wetland, their relative rates and sensitivity will be different, depending on type of wetland and nutrient condition. Thus, the application of the Everglades results to other wetlands should be viewed as starting point and caution should be exercised in selecting these indicators.

Several biogeochemical parameters and associated processes are affected by P loading to P limited areas of the Everglades. A summary of the results for the WCA-2a sites referred as impacted and reference conditions are describe below (Reddy et al. 1999). Total and bicarbonate extractable P were higher in the detrital layer and soils of the impacted site than the unimpacted site. The C:P ratio of detritus and soils decreases by over 50%

TABLE 2B: BACKGROUND CONCENTRATIONS (STANDARD ERROR) SELECTED BIOGEOCHEMICAL INDICATORS IN SURFACE SOIL (0–3 CM) COMPONENT OF VARIOUS HYDROLOGIC UNITS OF THE EVERGLADES. THE PMN AND PMP REFER TO POTENTIALLY MINERALIZABLE N AND P, RESPECTIVELY. (WRIGHT, ET AL. 2002)

Parameter	Units	WCA -1	(SE)	WCA -2A		WCA -3A		TS	
SOIL (0-3 CM)									
Loss on Ignition	%	91	(0)	72	(3)	90	(1)	20	(2)
Extractable C	g C kg^{-1}	3	(0)	3	(0)	5	(1)	2	(0)
Extractable NH_4-N	mg N kg^{-1}	116	(5)	112	(14)	109	(6)	29	(3)
Labile P	mg P kg^{-1}	2	(1)	1	(0)	8	(3)	1	(0)
Total P	mg P kg^{-1}	223	(10)	358	(27)	360	(10)	120	(11)
Total inorganic P	mg P kg^{-1}	45	(3)	153	(16)	103	(7)	50	(4)
Total N	g N kg^{-1}	36	(1)	29	(2)	41	(1)	10	(1)
Total C	g C kg^{-1}	459	(7)	360	(13)	463	(3)	193	(8)
Microbial Biomass C	g C kg^{-1}	3	(1)	3	(0)	3	(1)	2	(0)
Microbial Biomass N	mg N kg^{-1}	194	(38)	294	(46)	597	(97)	317	(85)
Microbial Biomass P	mg P kg^{-1}	77	(7)	76	(8)	24	(6)	20	(3)
PMN	mg N kg^{-1} d^{-1}	27	(3)	35	(7)	24	(4)	9	(2)
PMP	mg P kg^{-1} d^{-1}	2	0()	4	(1)	4	(0)	2	(0)
Soil O_2 Demand	mg kg^{-1} hr^{-1}	14	(4)	21	(3)	33	(3)	15	(5)
CH_4 Production	mg C kg^{-1} d^{-1}	-		-		105	(13)	21	(2)
Aerobic CO_2 Prod.	mg $kg^{-1}h^{-1}$	13	(5)	18	(6)	-		-	
Anaerobic CO_2 Prod.	mg $kg^{-1}h^{-1}$	10	(2)	13	(3)	-		-	

as a consequence of P inputs into the system. Clearly, P loading has enriched the soil forms of P. Microbial biomass (MB) C, N and P were also higher in the detrital layers and surface soils (0–10 cm depth) in P enriched areas. Phosphorus to microbial biomass, expressed as the ratio of MBP/P_{total} in the detrital layer, decreased from 27% to 16% in the impacted area. A similar 50% decrease in MBP/P_{total} was observed in the 0–10 cm soil interval. Increased MB has led, in turn, to higher rates of microbially mediated processes that regulate the biogeochemical cycling of C, N and P. Breakdown of organic matter had increased, evidenced by greater microbial respiration rates and higher activity of some extracellular enzymes. The net mineralization rates or releases of inorganic N and P were higher in soils and detrital layers at the elevated P site, increasing nutrient availability to higher plants. Nitrogen fixation, nitrification and denitrification rates also increased in the im-

Biogeochemical Indicator/Process	Detrital/Soil layer	Impact Index log [TS/RS]	Relative sensitivity to phosphorus loading
APA	D	–1.0 to –0.50	High
C/P ratio	D, S		
SIPM	S		
(SIPM/MBP)	D, S	–0.50 to –0.25	Medium
(MBP/TP)	S		
APA	S		
(MBP/TP)	D		
Ash content	D	–0.25 to –0.10	Low
Metabolic quotient	D		
Arylsulfatase	D		
Ash content	S		
Total C and N	D, S		
Metabolic quotient	S		
C/N ratio	D, S		
Extractable NH_4-N	S	–0.10 to 0.10	Negligible
SIPM	D		
PMP	S		
General aerobes	S		
Protease	S		
Protease	D		
Aerobicmicrobial respiration	D, S		
MBC	S		
MBN	D, S		
Extractable NH_4-N	D	0.10 to 0.25	Low
MBN/N total	D, S		
Nitrification	D		
Arylsulfatase	S		
Phenol oxidase	D, S		
		—	
Total P	S		
B-D glucosidase	S		
MBC	D		
Anaerobic microbial respiration	D, S		
PMN, SINM	D, S	0.25 to 0.50	Medium
Nitrification	D		
N_2 fixation	D		
DEA	D		
SINM/MBN	D, S		
MBP	D		
Total P	D		
B-D glucosidase	D		
N_2-fixation	D	0.50 to 1.0	High
SINM	D		
Bicarbonate extractable P	D, S		
PMP	D		

TABLE 3: IMPACT INDICES AND RELATIVE SENSITIVITY OF VARIOUS BIOGEOCHEMICAL PROCESSES/INDICATORS MEASURED IN DETRITAL AND SOIL LAYERS AT IMPACTED AND REFERENCE SITES IN THE EVERGLADES. D = DETRITAL LAYER AND S = 0–10 CM SOIL (REDDY, ET AL. 1999).

Biogeochemical Indicator/Process	Detrital/Soil layer	Impact Index log [TS/RS]	Relative sensitivity to phosphorus loading
Anaerobes*			
Acetate producers	S		
H_2 consumers	S		
CO_2 consumers	S	>1.0	Very High
Methanogens	S		
Sulfate reducers	S		

*Anaerobes were not measured in the detrital layer.

APA = alkaline phosphatase activity; C/P = carbon to phosphorus mass ratio; SIPM = substrate induced organic P mineralization; MBP = microbial biomass P; PMP = potentially mineralizable P; MBC = microbial biomass C; MBN = microbial biomass N; PMN = potentially mineralizable N; SINM = substrate induced organic N mineralization; and DEA = denitrification enzyme activity

pacted area. Overall, P loading increased the size of the microbial pool and organic matter turnover rate, which led to a greater release of inorganic N and P, further driving eutrophication.

These indicators were useful in evaluating impacts of nutrient loading on the ecosystem's health and depended on an indicator's natural variability within the system. Uncertainty in evaluation was caused by spatial variability, as well as the dynamic nature of many of the processes, complicating the extrapolation of laboratory results to field conditions. Temporal variation in these indicators introduced additional uncertainty in the interpretation of results. In an ecosystem with distinct gradients, impacts were described by using the relationship developed between biogeochemical processes and associated easily measurable parameters. However, these relationships needed to be based on data collected on sites having a wide range of physical, chemical and biological properties, and loading impacts.

For comparison purposes, a simple impact index was calculated in the Everglades for each process or parameter measured:

$$\text{Impact index} = \log [TS/RS]$$

Where [TS] is the rate or concentration of a parameter measured at an impacted or test site; and [RS] is the rate or concentration of a parameter measured at a reference site. The log [TS/RS] provided an index value of zero, which indicated no change, a negative value indicated a decrease and a positive value indicated an increase. For example, a value of "1" represented a ten-fold change in concentration of a parameter or rate of a process at the impacted site, relative to the reference site. This approach allowed the ranking of the parameters or processes most affected by nutrient loading/disturbance. Impact indices were viewed in the context of spatial variability within impacted and unimpacted sites. Field replicates may vary in relatively small areas ($<m^2$) and is process or parameter dependent. For example, enzyme activities in